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(54) Title: LIPID DELIVERY VEHICLE

(57) Abstract: The claimed invention is to a lipid delivery vehicle for eliciting a tolerogenic immune response in a subject, comprising nuclear factor-KB (NF-KB) inhibitor and a peptide antigen, wherein the lipid delivery vehicle comprises 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG).

“Lipid Delivery Vehicle”**Related application data**

[0001] The present application claims priority from Australian Patent Application No. 2023904233 filed on 22 December 2023 entitled “Lipid Delivery Vehicle”. The entire contents of which is hereby incorporated by reference.

Sequence listing

[0002] The present application is filed with a Sequence Listing, which has been submitted in electronically in XML format and is hereby incorporated by reference in its entirety. Said XML copy, created on 21 December 2024, is named “800861PCT sequence listing” and is 76,991 bytes in size.

Technical field

[0003] The present disclosure generally relates to a lipid delivery vehicle and uses thereof.

Background

[0004] Autoantigen-specific immunological tolerance induction (ASITI) is a therapeutic platform that controls both cellular and humoral immune responses in autoimmune diseases by re-establishing antigen-specific tolerance without impairing patients’ normal immunity. Generally, ASITI comprises the co-delivery of an immunomodulator and a peptide antigen encapsulated in a liposome, to antigen presenting cells (APCs) including dendritic cells (DC) in an attempt to induce antigen-specific induction of T cell tolerance.

[0005] However, strategies for liposome-based co-delivery of an immunomodulator and a peptide antigen face several translational hurdles to realise clinical benefit, including difficulties in scale-up production, long-term storage stability, liposomal stability upon exposure to plasma and the specificity of delivery to target tissues or cells.

[0006] An important aspect to consider when building an appropriate liposome-based delivery vehicle is the level of protection that the vehicle provides the encapsulated active pharmaceutical ingredients (APIs), as this will influence the longevity and integrity of the payload at the targeted tissue. Unprotected peptide may be degraded by exposure to tissue or blood proteases so that it is no longer an effective tolerogen. It is also important to protect the patient from the peptides until the payload is delivered and, ideally, autoantigenic peptides should be encapsulated within the delivery vehicle to avoid possible triggering of immune-complex-mediated immune responses.

[0007] Furthermore, residual unencapsulated peptide or surface-adsorbed peptide may lead to false assumptions about the concentration of liposome-encapsulated peptide, which is available for uptake by the immune target draining lymph node (dLN) DCs after subcutaneous administration. Prior to scale-up of methods to encapsulate a peptide in a liposome-based delivery

vehicle, free peptide is generally removed after liposome production by dialysis against HEPES buffer. However, at scale, peptide is typically and preferably removed by trans-flow filtration, which subjects liposomes to greater sheer stress, and surface-adsorbed peptide may not remain with the liposomes.

[0008] When contemplating lipid-based delivery vehicles for the growing market of subcutaneous delivery of APIs, plasma stability, storage stability, and retention of the APIs in the liposome prior to delivery of the payload is of critical importance. The leaching of APIs from lipid-based delivery vehicles is a known problem in the field, and placebo-controlled trials have shown that L- α -egg phosphatidylcholine (EPC) and L- α -egg phosphatidylglycerol (EPG) liposomes (i.e., DEN-181) release a substantial quantity of the encapsulated calcitriol immunomodulator into the plasma. The quantity of peptide released *in vivo* from these liposomes is unknown. The difficulties associated with the development of stable liposomes for subcutaneously administered APIs are markedly different to those associated with, for example, nasally or orally delivered APIs, which must navigate the unique challenges presented by the mucous membranes and the GI tract, respectively.

[0009] Thus, there remains a need for lipid delivery vehicles with improved pharmacokinetic profiles, especially for subcutaneous delivery, and in particular improved *in vivo* stability, distribution and dLN DC uptake and retention of lipid delivery vehicle, and the encapsulated peptide and immunomodulator.

[0010] However, in view of the vast number of suitable components that can be used in the construction of lipid-based delivery vehicles, the difficulties in extrapolating results across formulations for different administration routes, and the particular challenges of using peptide APIs, it is almost impossible to predict which lipids will combine to make a lipid-based delivery vehicle that satisfies all of these requirements.

Summary

[0011] In producing the present invention, the inventors unexpectedly identified that the combination of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG) may advantageously enhance the stability of the lipid delivery vehicle leading to improved peptide retention within the vehicle, both in storage and in plasma. In some forms of the disclosure, the inventors also found that lipid delivery vehicles comprising the combination of POPC and POPG may result in improved liposome targeting to, and uptake by, the draining lymph node (dLN) antigen presenting cells, including dendritic cells (DCs), improved peptide retention in dLNS and reduced off targeting effects (e.g., lower peptide retention in the spleen and liver). The inventors also identified that inclusion of a PEGylated lipid may further improve the stability of the lipid delivery vehicle and retention of peptide. The inventors also identified that the inclusion of POPC and POPG advantageously enhances

suppressive effects of the lipid delivery vehicle on dLN DCs. It has been further identified that construction at room temperature may improve the stability of the lipid delivery vehicle and retention of peptide. In some forms of the disclosure, the inventors also found that lipid delivery vehicles comprising the combination of POPC, POPG, a PEGylated lipid and calcitriol may result in improved targeting to, and uptake by, the dLNs and reduced off targeting effects (e.g., lower uptake by the liver). Based on these findings, the inventors have produced improved lipid delivery vehicles for the co-delivery of peptide antigens and immunomodulators.

[0012] Accordingly, the present disclosure provides a lipid delivery vehicle comprising a nuclear factor- κ B (NF- κ B) inhibitor and a peptide antigen, wherein the lipid delivery vehicle comprises 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG).

[0013] In one example, the nuclear factor- κ B (NF- κ B) inhibitor and peptide antigen are encompassed by or encapsulated within the lipid delivery vehicle.

[0014] The present disclosure also provides a lipid delivery vehicle comprising a nuclear factor- κ B (NF- κ B) inhibitor and a peptide antigen, wherein the lipid delivery vehicle comprises 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG) and a polyethylene glycol (PEG)ylated lipid.

[0015] The present disclosure also provides a population of lipid delivery vehicles disclosed herein, wherein the population of the lipid delivery vehicles has a polydispersity index of less than 0.20.

[0016] The present disclosure also provides a pharmaceutical composition comprising a lipid delivery vehicle disclosed herein and a pharmaceutically acceptable carrier.

[0017] The present disclosure also provides a pharmaceutical composition for subcutaneous delivery comprising the lipid delivery vehicle disclosed herein and a pharmaceutically acceptable carrier.

[0018] The present disclosure also provides a lipid delivery vehicle disclosed herein or a pharmaceutical composition disclosed herein for use in therapy.

[0019] The present disclosure also provides a method for eliciting a tolerogenic immune response in a subject, comprising administering to the subject a lipid delivery vehicle disclosed herein or a pharmaceutical composition disclosed herein to the subject.

[0020] The present disclosure also provides the use of a lipid delivery vehicle disclosed herein in the manufacture of a medicament for eliciting a tolerogenic immune response in a subject.

[0021] The present disclosure also provides a method for treating and/or preventing an autoimmune disease, disorder or condition in a subject, comprising administering to the subject a

lipid delivery vehicle disclosed herein or a pharmaceutical composition disclosed herein to the subject.

[0022] The present disclosure also provides the use of a lipid delivery vehicle disclosed herein in the manufacture of a medicament for treating and/or preventing an autoimmune disease, disorder or condition in a subject.

[0023] Particularly preferred embodiments are described herein, including in the independent claims.

Brief description of the drawings

[0024] The following figures form part of the present specification and are included to further demonstrate certain embodiments of the present disclosure. The disclosure may be better understood by reference to one or more of these figures in combination with the detailed description of specific embodiments presented herein. It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the above-described embodiments, without departing from the broad general scope of the present disclosure. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

[0025] **Figure 1** illustrates a Design of Experiments (DoE) model of the initial microfluidic experiments that were designed to identify liposome compositions that would satisfy the size, Polydispersity Index (PDI) and charge criteria with optimal peptide encapsulation.

[0026] **Figure 2** illustrates size exclusion columns used for size exclusion chromatography (SEC). (A) After incubation of liposomes in plasma or phosphate-buffered saline (PBS), the mixture is separated by SEC into fractions. (B) These fractions are then assessed for particle concentration using nanoparticle tracking analysis (NTA). (C) Liposome samples A and B were recovered in fractions 6-11, while the plasma in PBS (sample C) contained very few nanoparticles. (D) Peptide concentration, measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS), identified peaks in fractions 6-11 and 19-22. (E) When quantified, the majority of the peptide was recovered in fractions 6-11, with a small amount in fractions 12-22.

[0027] **Figure 3** illustrates (A) NTA for SEC assay and the (B) size distribution of particles.

[0028] **Figure 4** illustrates biodistribution of liposomes containing POPC/POPG/0.5% PEG/calcitriol/peptide (ASITI-201), EPC/EPG/calcitriol/peptide (DEN181) or POPC/POPG/0.5%PEG (empty) in the (A) skin injection site, (B) inguinal dLN, (C) axillary dLN and (D) liver of naive mice.

[0029] **Figure 5** illustrates the peptide biodistribution after free peptide or liposomes containing POPC/POPG/ PEG/ PI₃₃₋₆₃/calcitriol. (ASITI-201) (A) Radiochemical purity of ⁸⁹ZrPI₃₃₋₆₃. (B) Quantification of ⁸⁹ZrPI₃₃₋₆₃ peptide biodistribution over time in this experiment. (C) Positron

emission tomography-computed tomography (PET-CT) of whole mice showing ^{89}Zr signal, 2 days after liposome or free peptide administration subcutaneous to the left flank.

[0030] Figure 6 illustrates the antigen presenting cell uptake of DiI-labelled liposomes in (A) skin, (B) liver, (C) dLN and (D) spleen of naive mice. The proportion of DiI⁺ dendritic cells, B cells and monocytes at the skin injection site, dLN (combined inguinal and axillary), liver and spleen, 2 days post subcutaneous injection of 100 μL of DiI-labelled DEN-181 or ASITI-201. PBS control denotes background fluorescence in the DiI channel.

[0031] Figure 7 illustrates the *in vivo* biodistribution of free ^{89}Zr PI₃₃₋₆₃ peptide or liposomes containing POPC/POPG/PEG/ ^{89}Zr PI₃₃₋₆₃/calcitriol or EPC/EPG/ ^{89}Zr PI₃₃₋₆₃/calcitriol in the (A) left inguinal dLN, (B) right inguinal dLN, (C) left axillary dLN, (D) right axillary dLN, after subcutaneous injection at the base of tail, (E) injection site, (F) day 3 biodistribution and (G) day 11 biodistribution.

[0032] Figure 8 illustrates (A) experimental process comparing liposome uptake by and impact on antigen presenting cells (APCs) and on antigen-reactive CD4⁺ T cells after multiple dosing in antigen-primed mice, (B) uptake by APCs in dLNs and (C) uptake by APCs in spleen.

[0033] Figure 9 illustrates flow cytometry analysis of impact of liposome uptake on (A) CD11c⁺ myeloid DCs in dLN, (B) plasmacytoid DCs in dLN, (C) memory B cells in dLN, (D) germinal centre B cells in dLN, (E) aggrecan stimulated CD4⁺ T cell CD154 in spleen, (F) CD154⁺ T cell IFN- γ ⁺ in spleen and (G) CD154⁺ T cell IFN- γ ⁺ TNF⁺ in spleen.

[0034] Figure 10 illustrates the *in vivo* biodistribution of liposomes containing POPC/POPG/PEG/PI₃₃₋₆₃/[^3H]calcitriol or EPC/EPG/PI₃₃₋₆₃/[^3H]calcitriol at 1 and 72 hours in the (A) injection site, (B) both inguinal dLNs, (C) both axillary dLNs, (D) blood, (E) liver, (F) spleen, (G) kidney and (H) gut/fecal after subcutaneous injection at the base of tail. * denotes group differences and * with square bracket denotes differences over time.

KEY TO SEQUENCE LISTING

SEQ ID NO: 1	Amino acid sequence of Collagen ₂₅₉₋₂₇₃ II
SEQ ID NO: 2	Amino acid sequence of Proinsulin ₃₃₋₆₃
SEQ ID NO: 3	Amino acid sequence of Ovalbumin ₃₂₃₋₃₃₉
SEQ ID NO: 4	Amino acid sequence of Aggrecan ₈₄₋₁₀₃ Cit93
SEQ ID NO: 5	Amino acid sequence of Ro60 ₄₀₁₋₄₂₅
SEQ ID NO: 6	Amino acid sequence of Vimentin ₄₁₋₈₅
SEQ ID NO: 7	Amino acid sequence of Vimentin ₄₁₋₈₅ Cit64
SEQ ID NO: 8	Amino acid sequence of Vimentin ₃₈₋₈₈ Cit64
SEQ ID NO: 9	Amino acid sequence of MPO ₄₃₅₋₄₅₄
SEQ ID NO: 10	Amino acid sequence of MPO ₄₄₇₋₄₅₉

SEQ ID NO: 11	Amino acid sequence of AggreCan ²⁰⁰⁻²⁴⁴ Cit ²¹⁰ ,Cit ²³⁰ ,Cit ²³⁵
SEQ ID NO: 12	Amino acid sequence of AggreCan ³⁻²⁰
SEQ ID NO: 13	Amino acid sequence of AggreCan ¹⁵³⁻¹⁶⁸
SEQ ID NO: 14	Amino acid sequence of AggreCan ¹⁶¹⁻¹⁷⁸
SEQ ID NO: 15	Amino acid sequence of AggreCan ²⁰⁰⁻²¹⁵
SEQ ID NO: 16	Amino acid sequence of AggreCan ²²⁵⁻²⁴⁴
SEQ ID NO: 17	Amino acid sequence of AggreCan ²⁹⁸⁻³¹³
SEQ ID NO: 18	Amino acid sequence of AggreCan ⁴⁷⁷
SEQ ID NO: 19	Amino acid sequence of AggreCan ⁵²⁰
SEQ ID NO: 20	Amino acid sequence of AggreCan ⁵⁵³
SEQ ID NO: 21	Amino acid sequence of AggreCan ⁵⁶⁸
SEQ ID NO: 22	Amino acid sequence of AggreCan ⁵⁷⁹
SEQ ID NO: 23	Amino acid sequence of AggreCan ⁶²¹
SEQ ID NO: 24	Amino acid sequence of AggreCan ⁶⁸⁴
SEQ ID NO: 25	Amino acid sequence of TSH ²⁰⁻³⁵
SEQ ID NO: 26	Amino acid sequence of TSH ³⁰⁻⁴⁹
SEQ ID NO: 27	Amino acid sequence of TSH ⁴⁴⁻⁶²
SEQ ID NO: 28	Amino acid sequence of TSH ⁵⁷⁻⁷⁵
SEQ ID NO: 29	Amino acid sequence of TSH ⁷⁰⁻⁸⁸
SEQ ID NO: 30	Amino acid sequence of TSH ⁸³⁻¹⁰²
SEQ ID NO: 31	Amino acid sequence of TSH ⁹⁷⁻¹¹²
SEQ ID NO: 32	Amino acid sequence of TSH ¹⁰⁹⁻¹²⁴
SEQ ID NO: 33	Amino acid sequence of TSH ¹¹⁹⁻¹³⁷
SEQ ID NO: 34	Amino acid sequence of TSH ¹³²⁻¹⁵⁰
SEQ ID NO: 35	Amino acid sequence of TSH ¹⁴⁵⁻¹⁶³
SEQ ID NO: 36	Amino acid sequence of TSH ¹⁵⁸⁻¹⁷⁶
SEQ ID NO: 37	Amino acid sequence of TSH ¹⁷²⁻¹⁸⁶
SEQ ID NO: 38	Amino acid sequence of TSH ¹⁸³⁻¹⁹⁸
SEQ ID NO: 39	Amino acid sequence of TSH ¹⁹⁵⁻²¹⁰
SEQ ID NO: 40	Amino acid sequence of TSH ²⁰⁷⁻²²²
SEQ ID NO: 41	Amino acid sequence of TSH ²¹⁷⁻²³²
SEQ ID NO: 42	Amino acid sequence of TSH ²²⁷⁻²⁴²
SEQ ID NO: 43	Amino acid sequence of TSH ²³⁷⁻²⁵²
SEQ ID NO: 44	Amino acid sequence of TSH ²⁴⁸⁻²⁶³
SEQ ID NO: 45	Amino acid sequence of TSH ²⁵⁸⁻²⁷⁷

SEQ ID NO: 46	Amino acid sequence of TSH ₂₇₂₋₂₉₁
SEQ ID NO: 47	Amino acid sequence of TSH ₂₈₆₋₃₀₅
SEQ ID NO: 48	Amino acid sequence of TSH ₃₀₁₋₃₂₀
SEQ ID NO: 49	Amino acid sequence of TSH ₃₁₅₋₃₃₄
SEQ ID NO: 50	Amino acid sequence of TSH ₃₂₉₋₃₄₈
SEQ ID NO: 51	Amino acid sequence of TSH ₃₄₃₋₃₆₂
SEQ ID NO: 52	Amino acid sequence of TSH ₃₅₇₋₃₇₆
SEQ ID NO: 53	Amino acid sequence of TSH ₃₇₁₋₃₉₀
SEQ ID NO: 54	Amino acid sequence of TSH ₃₈₅₋₄₀₄
SEQ ID NO: 55	Amino acid sequence of TSH ₃₉₉₋₄₁₈
SEQ ID NO: 56	Amino acid sequence of Tenascin C ₈₇₁₋₈₈₅ Cit876,Cit877
SEQ ID NO: 57	Amino acid sequence of Tenascin C ₁₀₁₂₋₁₀₂₆ Cit1014,Cit1016
SEQ ID NO: 58	Amino acid sequence of Tenascin C ₁₆₃₃₋₁₆₄₇ Cit1637
SEQ ID NO: 59	Amino acid sequence of Tenascin C ₂₀₆₇₋₂₀₈₁ Cit2073,Cit2077
SEQ ID NO: 60	Amino acid sequence of HIP1 (PI ₄₀₋₄₇ -IAPP ₇₄₋₈₀)
SEQ ID NO: 61	Amino acid sequence of HIP2 (PI ₄₀₋₄₇ -IAPP ₂₃₋₂₉)
SEQ ID NO: 62	Amino acid sequence of HIP3 (PI ₄₀₋₄₇ -NPY ₆₈₋₇₄)
SEQ ID NO: 63	Amino acid sequence of HIP4
SEQ ID NO: 64	Amino acid sequence of HIP5 (insulin-BDC-2.5)
SEQ ID NO: 65	Amino acid sequence of HIP6 (insulin-BDC-6.9)
SEQ ID NO: 66	Amino acid sequence of Vimentin ₅₃₋₈₅ Cit64
SEQ ID NO: 67	Amino acid sequence of Vimentin ₆₀₋₈₅ Cit64
SEQ ID NO: 68	Amino acid sequence of human Vimentin
SEQ ID NO: 69	Amino acid sequence of AggreCAN ₈₉₋₁₀₃
SEQ ID NO: 70	Amino acid sequence of human desmoglein 2 (hDSG2)
SEQ ID NO: 71	Amino acid sequence of Vimentin ₅₉₋₇₁

Detailed description

General Techniques and Definitions

[0035] Unless specifically defined otherwise, all technical and scientific terms used herein shall be taken to have the same meaning as commonly understood by one of ordinary skill in the art (e.g. in genomics, immunology, molecular biology, immunohistochemistry, biochemistry, oncology, and pharmacology).

[0036] The present disclosure is performed using, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA technology and immunology. Such procedures are described, for example in Sambrook, Fritsch & Maniatis, Molecular Cloning:

A Laboratory Manual, Cold Spring Harbor Laboratories, New York, Fourth Edition (2012), whole of Vols I, II, and III; DNA Cloning: A Practical Approach, Vols. I and II (D. N. Glover, Second Edition., 1995), IRL Press, Oxford, whole of text; Oligonucleotide Synthesis: A Practical Approach (M. J. Gait, ed, 1984) IRL Press, Oxford, whole of text, and particularly the papers therein by Gait, pp 1-22; Atkinson *et al*, pp 35-81; Sproat *et al*, pp 83-115; and Wu *et al*, pp 135-151; 4. Nucleic Acid Hybridization: A Practical Approach (B. D. Hames & S. J. Higgins, eds., 1985) IRL Press, Oxford, whole of text; Immobilized Cells and Enzymes: A Practical Approach (1986) IRL Press, Oxford, whole of text; Perbal, B., A Practical Guide to Molecular Cloning (1984), Current Protocols in Immunology, Eds. (John Wiley & Sons Inc, 1999-2023). and Methods In Enzymology (S. Colowick and N. Kaplan, eds., Academic Press, Inc.), whole of series.

[0037] Those skilled in the art will appreciate that the present disclosure is susceptible to variations and modifications other than those specifically described. It is to be understood that the disclosure includes all such variations and modifications. The disclosure also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features. Thus, each feature of any particular example or embodiment of the present disclosure may be applied *mutatis mutandis* to any other example or embodiment of the present disclosure.

[0038] The present disclosure is not to be limited in scope by the specific embodiments described herein, which are intended for the purpose of exemplification only. Functionally equivalent products, compositions and methods are clearly within the scope of the disclosure, as described herein.

[0039] Throughout this specification, unless specifically stated otherwise or the context requires otherwise, reference to a single step, composition of matter, group of steps or group of compositions of matter shall be taken to encompass one and a plurality (i.e. one or more) of those steps, compositions of matter, groups of steps or group of compositions of matter.

[0040] The terms “from”, “to” and “between”, when indicating a range, shall be understood to mean the range is inclusive of the recited lower and upper values. For example, “x is an integer from 0 to 6” shall be understood as including the situation in which x is not present (x is 0), that in which x is 6, as well as each whole number integer value in between, i.e., x is 1, 2, 3, 4, or 5.

[0041] As used herein, the singular forms of “a”, “and” and “the” include plural forms of these words, unless the context clearly dictates otherwise.

[0042] The term “and/or”, e.g., “X and/or Y” shall be understood to mean either “X and Y” or “X or Y” and shall be taken to provide explicit support for both meanings or for either meaning.

[0043] Throughout this specification, the word “comprise” or variations such as “comprises” or “comprising” will be understood to imply the inclusion of a stated element, integer or step, or

group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

[0044] The term “about” in relation to a numerical value x is optional and means, for example, any number within 1, 5 or 10% of the referenced number. The term “about” also encompasses the exact number recited.

[0045] By “consisting essentially of”, in the context of: (a) an amino acid sequence, is meant the recited amino acid sequence together with an additional one, two or three amino acids at the N- and/or C-terminus thereof; or (b) a nucleic acid sequence, is meant the recited nucleic acid sequence together with an additional one, two or three nucleic acids at the 5' and/or 3' thereof.

[0046] All computer programs, algorithms, patent and scientific literature referred to herein is incorporated herein by reference.

[0047] Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present disclosure as it existed before the priority date of each of the appended claims.

Lipid delivery vehicle

[0048] In work leading up to the invention, the inventors sought to produce a lipid delivery vehicle with an improved pharmacokinetic profile (e.g., storage stability, plasma stability), enhanced targeting of, and uptake by the dLNs and/or reduced off-targeting effects. Consequently, the inventors have developed a lipid delivery vehicle comprising 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG) with improved pharmacokinetic profile (e.g., stability) in plasma and the lymphatic system. In some forms of the disclosure, the inventors also identified that this lipid delivery vehicle improves targeting of the dLNs and/or reduces off-targeting effects (e.g., reduced uptake by the liver).

[0049] Accordingly, the present disclosure provides a lipid delivery vehicle comprising a nuclear factor- κ B (NF- κ B) inhibitor and a peptide antigen, wherein the lipid delivery vehicle comprises 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG).

[0050] As used herein, the term “lipid delivery vehicle” shall be understood to refer to lipid-based particles having at least one dimension on the order of nanometers (e.g., 1 – 1,000 nm). Lipid delivery vehicles are formulated in a composition for delivery of molecules (e.g., APIs such as peptide antigens and/or nuclear factor- κ B inhibitors) to a desired target such as a cell, tissue, organ, tumor, and the like. The lipid delivery vehicle may be selected from, but not limited to, liposomes or lipid vesicles, where an aqueous volume is encapsulated by one or more amphipathic lipid bilayers (e.g., single; unilamellar or multiple; multilamellar), micelle-like lipid nanoparticles

having a non-aqueous core and solid lipid nanoparticles, wherein solid lipid nanoparticles lack lipid bilayers.

[0051] When the lipid delivery vehicle is a liposome or lipid vesicle loaded for the delivery of one or more APIs, the APIs may be encapsulated by one or more amphipathic lipid bilayers (i.e., buried within the layers), or encapsulated within the vehicle (i.e., encapsulated within the aqueous volume internal to the vehicle), or a combination of both. For the purposes of the disclosure, peptide antigens and/or nuclear factor- κ B inhibitors that are “encapsulated within” the lipid delivery vehicle is meant to encompass any and all peptide antigens and/or nuclear factor- κ B inhibitors that are associated with the lipid delivery vehicle, with the exception of (a) any peptide antigen and/or nuclear factor- κ B inhibitor that is solely bound to, or associated with, the surface of the lipid delivery vehicle, and (b) any peptide antigen and/or nuclear factor- κ B inhibitor that is merely present in a composition comprising a loaded lipid delivery vehicle (i.e., a lipid delivery vehicle with encapsulated APIs) but is not bound to, or associated with, lipid delivery vehicle.

[0052] In embodiments, the term “lipid delivery vehicle” may refer to a lipid particle formed from POPC and POPG, and an optional PEGylated lipid, as the only lipid-containing components.

[0053] In embodiments, the lipid delivery vehicle does not comprise any further phospho-lipids other than POPC and POPG. In embodiments, the lipid delivery vehicle does not comprise any non-PEGylated lipids other than POPC and POPG.

[0054] In embodiments, the lipid delivery vehicle consists or consists essentially of POPC, POPG, and an optional PEGylated lipid as the only lipid components of the lipid delivery vehicle.

[0055] The lipid delivery vehicle described herein may have a mean diameter of about 75 nm to about 250 nm, or about 100 nm to about 200 nm, or about 110 nm to about 200 nm, or about 120 nm to about 200 nm, or about 100 nm to about 190 nm, or about 100 nm to about 160 nm, or about 100 nm to about 170 nm, or about 100 nm to about 160 nm, or about 100 nm to about 150 nm, or about 100 nm to about 140 nm, or about 100 nm to about 130 nm, or about 110 nm to about 190 nm, or about 110 nm to about 180 nm, or about 110 nm to about 170 nm, or about 110 nm to about 160 nm, or about 110 nm to about 150 nm, or about 110 nm to about 140 nm, or about 110 nm to about 130 nm. The lipid delivery vehicle described herein may have a mean diameter of about 90 nm to about 160 nm. For example, the lipid delivery vehicle described herein may comprise a mean diameter of about 100 nm, or about 110 nm, or about 120 nm, or about 130 nm, or about 140 nm, or about 150 nm, or about 160 nm, or about 170 nm, or about 180 nm, or about 190 nm, or about 200 nm. The lipid delivery vehicle described herein may have a mean diameter of about 60 nm to about 180 nm. The lipid delivery vehicle described herein may have a mean diameter of about 80 nm to about 150 nm. The lipid delivery vehicle described herein may have a mean diameter of about 110 nm to about 150 nm. The lipid delivery vehicle described herein may have

a mean diameter of about 80 nm to about 100 nm. For example, the lipid delivery vehicle described herein may comprise a mean diameter of about 60 nm, or about 70 nm, or about 80 nm, or about 90 nm, or about 100 nm, or about 110 nm, or about 120 nm, or about 130 nm, or about 140 nm, or about 150 nm, or about 160 nm, or about 170 nm, or about 180 nm.

[0056] The diameter of the lipid delivery vehicle may be measured by dynamic light scattering (DLS), transmission electron microscopy (TEM), scanning electron microscopy (SEM), or other methods such as are known in the art.

[0057] Electrophoretic light scattering may be used to characterise the surface charge of the lipid delivery vehicle at a specified pH, for example physiological pH. The surface charge, or the zeta potential, is a measure of the magnitude of electrostatic repulsion/attraction between particles in the lipid delivery vehicle suspension. The surface charge of the lipid delivery vehicle at physiological pH (i.e., between pH 7.0 and 7.4) may be negative. For example, the surface charge may be less than 0 mV, or less than -5 mV, or less than -10 mV, or less than -15 mV, or less than -20 mV, or less than -25 mV, or less than -30 mV, or less than -40 mV, or less than -50 mV. The surface charge of the lipid delivery vehicle at physiological pH (i.e., between pH 7.0 and 7.4) may be less than -20 mV. Alternatively, the surface charge of the lipid delivery vehicle at physiological pH (i.e., between pH 7.0 and 7.4) may be less than -30 mV. Alternatively, the surface charge of the lipid delivery vehicle at physiological pH (i.e., between pH 7.0 and 7.4) may be less than -40 mV. Alternatively, the surface charge of the lipid delivery vehicle at physiological pH (i.e., between pH 7.0 and 7.4) may be less than -50 mV. Alternatively, the surface charge of the lipid delivery vehicle at physiological pH (i.e., between pH 7.0 and 7.4) may be between -10 mV and -50 mV. Alternatively, the surface charge of the lipid delivery vehicle at physiological pH (i.e., between pH 7.0 and 7.4) may be between -30 mV and -50 mV. Alternatively, the surface charge of the lipid delivery vehicle at physiological pH (i.e., between pH 7.0 and 7.4) may be between -40 mV and -50 mV.

[0058] Dynamic Light Scattering (“DLS”) may be used to characterise the polydispersity index (“PdI”) and size of a population of lipid delivery vehicles described herein. DLS measures the scattering of light that results from subjecting a sample to a light source. PdI, as determined from DLS measurements, represents the distribution of particle size (around the mean particle size) in a population, with a perfectly uniform population having a PdI of zero.

[0059] Populations of the lipid delivery vehicles described herein may be relatively homogenous. A polydispersity index may be used to indicate the homogeneity of a population of lipid delivery vehicles. A small (e.g., less than 0.2) polydispersity index generally indicates population with a narrow particle size distribution. A population of the lipid delivery vehicles described herein may have a polydispersity index from about 0 to about 0.25, such as 0.01, or 0.02, or 0.03, or 0.04, or

0.05, or 0.06, or 0.07, or 0.08, or 0.09, or 0.10, or 0.11, or 0.12, or 0.13, or 0.14, or 0.15, or 0.16, or 0.17, or 0.18, or 0.19, or 0.20, or 0.21, or 0.22, or 0.23, or 0.24, or 0.25. The polydispersity index of a population of the lipid delivery vehicles may be about 0 to about 0.20, or about 0.05 to about 0.20. The polydispersity index of a population of the lipid delivery vehicles may be about 0 to about 0.15.

[0060] Cryo-electron microscopy (“cryo-EM”) may be used to determine the particle size, morphology, and structural characteristics of a lipid delivery vehicle.

[0061] Lipid compositional analysis of the lipid delivery vehicles may be determined from liquid chromatography followed by charged aerosol detection (LC-CAD). This analysis may provide a comparison of the actual lipid content versus the theoretical lipid content.

[0062] The lipid delivery vehicle of the present disclosure may be selected from the group consisting of a liposome, a lipid nanoparticle, a lipid vesicle and a lipid-based particle. For example, the lipid delivery vehicle may be a liposome. Alternatively, the lipid delivery vehicle may be a lipid nanoparticle. The lipid delivery vehicle may be a lipid vesicle. The lipid delivery vehicle may also be a lipid-based particle.

Lipids

[0063] The lipid delivery vehicle described herein may comprise about 85 wt % to about 95 wt % of POPC. For example, the lipid delivery vehicle may comprise about 85 wt %, or about 86 wt %, or about 87 wt %, or about 88 wt %, or about 89 wt %, or about 90 wt %, or about 91 wt %, or about 92 wt %, or about 93 wt %, or about 94 wt %, or about 95 wt % of POPC.

[0064] Alternatively, the lipid delivery vehicle described herein may comprise about 85 mol % to about 95 mol % of POPC. For example, the lipid delivery vehicle may comprise about 85 mol %, or about 86 mol %, or about 87 mol %, or about 88 mol %, or about 89 mol %, or about 90 mol %, or about 91 mol %, or about 92 mol %, or about 93 mol %, or about 94 mol %, or about 95 mol % of POPC.

[0065] Alternatively, the lipid delivery vehicle described herein may comprise about 19 mg/mL to about 37 mg/mL of POPC. For example, the lipid delivery vehicle may comprise about 19 mg/mL, or about 20 mg/mL, or about 21 mg/mL, or about 22 mg/mL, or about 23 mg/mL, or about 24 mg/mL, or about 25 mg/mL, or about 26 mg/mL, or about 27 mg/mL, or about 28 mg/mL, or about 29 mg/mL, or about 30 mg/mL, or about 31 mg/mL, or about 32 mg/mL, or about 33 mg/mL, or about 34 mg/mL, or about 35 mg/mL, or about 36 mg/mL, or about 37 mg/mL of POPC.

[0066] The lipid delivery vehicle described herein may comprise about 5 wt % to about 15 wt % of POPG. For example, the lipid delivery vehicle may comprise about 5 wt %, or about 6 wt %, or about 7 wt %, or about 8 wt %, or about 9 wt %, or about 10 wt %, or about 11 wt %, or about 12 wt %, or about 13 wt %, or about 14 wt %, or about 15 wt % of POPG.

[0067] Alternatively, the lipid delivery vehicle described herein may comprise about 5 mol % to about 15 mol % of POPG. For example, the lipid delivery vehicle may comprise about 5 mol %, or about 6 mol %, or about 7 mol %, or about 8 mol %, or about 9 mol %, or about 10 mol %, or about 11 mol %, or about 12 mol %, or about 13 mol %, or about 14 mol %, or about 15 mol % of POPG.

[0068] Alternatively, the lipid delivery vehicle described herein may comprise about 2.4 mg/mL to about 3.7 mg/mL of POPG. For example, the lipid delivery vehicle may comprise about 2.4 mg/mL, or about 2.5 mg/mL, or about 2.6 mg/mL, or about 2.7 mg/mL, or about 2.8 mg/mL, or about 2.9 mg/mL, or about 3.0 mg/mL, or about 3.1 mg/mL, or about 3.2 mg/mL, or about 3.3 mg/mL, or about 3.4 mg/mL, or about 3.5 mg/mL, or about 3.6 mg/mL, or about 3.7 mg/mL of POPG.

[0069] In some forms of the disclosure, the inventors also identified that inclusion of a PEGylated lipid may improve targeting of the dLNs and/or reduce off-targeting effects (e.g., reduced uptake by the liver). Accordingly, the lipid delivery vehicle may further comprise a PEGylated lipid.

[0070] It will be apparent to the skilled person that reference to a PEGylated lipid is a lipid that has been modified with polyethylene glycol (PEG). Exemplary PEGylated lipids include, but are not limited to, PEG-modified phosphatidylethanolamines, PEG-modified phosphatidic acids, PEG-modified ceramides, PEG-modified dialkylamines, PEG-modified diacylglycerols, and PEG-modified dialkylglycerols. A suitable PEGylated lipid may include PEG-c-DOMG, PEG-DMG (e.g., 1,2-Dimyristoyl-sn-glycero-3-methoxypolyethylene glycol (DMG-PEG 2000K)), PEG-DLPE, PEG-DMPE, PEG-DPPC, a PEG-DSPE lipid and any combination thereof.

[0071] The average molecular weight of the PEG in the PEGylated lipid may be 5000 Da or less, or 4000 Da or less, or 3000 Da or less, or 2000 Da or less, or 1000 Da or less. For example, the average molecular weight of the PEG may be between about 500 Da to about 5000 Da or between about 1000 Da to about 4000 Da. The average molecular weight of the PEG may be about 2000 Da.

[0072] The lipid delivery vehicle described herein may comprise about 0.1 wt % to about 5 wt % of a PEGylated lipid. For example, the lipid delivery vehicle may comprise about 0.1 wt % to about 2 wt % of a PEGylated lipid. In one example, the lipid delivery vehicle may comprise about 0.5 wt % to about 5 wt % of a PEGylated lipid. In one example, the lipid delivery vehicle may comprise about 0.5 wt % to about 3 wt % of a PEGylated lipid. In one example, the lipid delivery vehicle may comprise about 0.1 wt %, or about 0.2 wt %, or about 0.3 wt %, or about 0.4 wt %, or about 0.5 wt %, or about 0.6 wt %, or about 0.7 wt %, or about 0.8 wt %, or about 0.9 wt %, or about 1 wt %, or about 1.1 wt %, about 1.2 wt %, or about 1.3 wt %, or about 1.4 wt %, or about 1.5 wt %, or about 1.6 wt %, or about 1.7 wt %, or about 1.8 wt %, or about 1.9 wt %, or about 2

wt %, or about 2.1 wt %, about 2.2 wt %, or about 2.3 wt %, or about 2.4 wt %, or about 2.5 wt %, or about 2.6 wt %, or about 2.7 wt %, or about 2.8 wt %, or about 2.9 wt %, or about 3 wt %, or about 3.1 wt %, about 3.2 wt %, or about 3.3 wt %, or about 3.4 wt %, or about 3.5 wt %, or about 3.6 wt %, or about 3.7 wt %, or about 3.8 wt %, or about 3.9 wt %, or about 4 wt %, or about 4.1 wt %, about 4.2 wt %, or about 4.3 wt %, or about 4.4 wt %, or about 4.5 wt %, or about 4.6 wt %, or about 4.7 wt %, or about 4.8 wt %, or about 4.9 wt %, or about 5 wt % of a PEGylated lipid.

[0073] Alternatively, the lipid delivery vehicle described herein may comprise about 0.1 mol % to about 5 mol % of a PEGylated lipid. For example, the lipid delivery vehicle may comprise about 0.1 mol %, or about 0.2 mol %, or about 0.3 mol %, or about 0.4 mol %, or about 0.5 mol %, or about 0.6 mol %, or about 0.7 mol %, or about 0.8 mol %, or about 0.9 mol %, or about 1 mol %, or about 2 mol %, or about 3 mol %, or about 4 mol %, or about 5 mol % of a PEGylated lipid.

[0074] Alternatively, the lipid delivery vehicle described herein may comprise about 0.1 mg/mL to about 1 mg/mL of a PEGylated lipid. For example, the lipid delivery vehicle may comprise about 0.1 mg/mL, or about 0.2 mg/mL, or about 0.3 mg/mL, or about 0.4 mg/mL, or about 0.5 mg/mL, or about 0.6 mg/mL, or about 0.7 mg/mL, or about 0.8 mg/mL, or about 0.9 mg/mL, or about 1 mg/mL of a PEGylated lipid.

[0075] Alternatively, the lipid delivery vehicle described herein may comprise about 0.3 mg/mL to about 2 mg/mL of a PEGylated lipid. For example, the lipid delivery vehicle may comprise about 0.3 mg/mL, or about 0.4 mg/mL, or about 0.5 mg/mL, or about 0.6 mg/mL, or about 0.7 mg/mL, or about 0.8 mg/mL, or about 0.9 mg/mL, or about 1 mg/mL, or about 1.1 mg/mL, or about 1.2 mg/mL, or about 1.3 mg/mL, or about 1.4 mg/mL, or about 0.5 mg/mL, or about 0.6 mg/mL, or about 1.7 mg/mL, or about 1.8 mg/mL, or about 1.9 mg/mL, or about 2 mg/mL of a PEGylated lipid

[0076] The lipid delivery vehicle may not comprise cholesterol.

Surfactants

[0077] Surfactants may be incorporated into, or used to fabricate, lipid delivery vehicles. Suitably, the surfactant may be a phosphoglyceride. Exemplary phosphoglycerides may include phosphatidylcholines, such as the naturally occurring surfactant, L- α -phosphatidylcholine dipalmitoyl (DPPC). The surfactants may advantageously improve surface properties of the lipid delivery vehicle by, for example, reducing particle-particle interactions, and may render the surface of the vehicles less adhesive. The use of surfactants endogenous to the lung may avoid the need for the use of non-physiologic surfactants.

[0078] Providing a surfactant on the surface of the lipid delivery vehicles may reduce the tendency of the particles to agglomerate due to interactions such as electrostatic interactions, Van der Waals

forces, and capillary action. The presence of the surfactant on the lipid delivery vehicle surface may provide increased surface rugosity (roughness), thereby improving aerosolization by reducing the surface area available for intimate particle-particle interaction.

[0079] Surfactants known in the art can be used including any naturally occurring surfactant. Other exemplary surfactants include phospholipids such as diphosphatidyl glycerol (DPPG) or phosphatidylethanolamine; fatty alcohols or fatty acids such as palmitic acid or oleic acid polyoxyethylene-9-lauryl ether; sorbitan esters such as sorbitan trioleate (Span 85); bile salts; and amphiphilic polymers such as poloxamers or proteins. Mixtures of surfactants may also be used.

Peptide Antigens

[0080] The term “peptide antigen” shall be understood to mean all, or part of, a protein or peptide, capable of eliciting an immune response in a vertebrate animal, especially a mammal. Such antigens may also be reactive with antibodies from animals immunised with that protein or peptide.

[0081] The term “autoantigen” shall be understood to refer to a particular subset of antigens derived from proteins or peptides are produced by a mammal, and recognised by said mammal’s immune system, to elicit a typically unwanted immune response.

[0082] The term “derived from” as used herein shall be understood to refer to an antigen consisting of a fragment or portion of the peptide from which it is derived, or comprising of a fragment or portion of the peptide, a sufficiently high level of sequence or structural homology with the peptide, and/or sharing a sufficiently high level of sequence or structural homology with a fragment or portion of the peptide, such that the antigen is capable eliciting an immune response relevant to the peptide.

[0083] By “immune response” it is meant the induction of a humoral or cell-mediated response in a subject. The humoral or cell-mediated response may be specific to the peptide antigen. Such a response may be detected and/or quantified by determining the induction of antibodies in the subject and/or cellular responses. A quantitative antibody measurement may be determined. Alternatively, or in addition, a qualitative antibody measurement may be determined. For example, but without limitation, a measure of one or more functional features of antibodies elicited in a subject to which the peptide antigen is administered, may be determined.

[0084] The term “protein” shall be taken to include a single polypeptide chain, i.e., a series of contiguous amino acids linked by peptide bonds or a series of polypeptide chains covalently or non-covalently linked to one another (i.e., a polypeptide complex). For example, the series of polypeptide chains can be covalently linked using a suitable chemical or a disulfide bond. Examples of non-covalent bonds include hydrogen bonds, ionic bonds, Van der Waals forces, and hydrophobic interactions.

[0085] The term “peptide” as used herein is intended to include compounds composed of amino acid residues linked by amide bonds. A peptide may be natural or unnatural, ribosome encoded or synthetically derived. Typically, a peptide will consist of between 2 and 200 amino acids. For example, the peptide may have a length in the range of 10 to 20 amino acids or 10 to 30 amino acids or 10 to 40 amino acids or 10 to 50 amino acids or 10 to 60 amino acids or 10 to 70 amino acids or 10 to 80 amino acids or 10 to 90 amino acids or 10 to 100 amino acids, including any length within said range(s). The terms “protein” and “peptide” are used interchangeably herein.

[0086] Various peptide antigens are associated with unwanted or deleterious immune responses exist. For example, the peptide antigen may be an autoantigen, an alloantigen or an allergen.

[0087] Examples of autoantigens may include, but are not limited to, peptide antigens derived from Ro (e.g., Ro₄₀₁₋₄₂₅), collagen type II (CII) (e.g., CII₂₅₉₋₂₇), proinsulin (PI) (e.g., PI₃₃₋₆₃), insulin, chromogranin, aggrecan (e.g., aggrecan₂₀₀₋₂₄₄), islet antigen 2 (IA2), glutamic acid decarboxylase 65-kilodalton isoform (GAD65), hybrid insulin peptides (HIPs), glycoprotein (gp70), nuclear antigens, lupus autoantigen, Smith, La, U1-RNP, fibrillin, histones, ribosomal proteins, pyruvate dehydrogenase dihydrolipoamide acetyltransferase (PCD-E2), hair follicle antigens, human tropomyosin isoform 5 (hTM5), human cartilage gp 39 (HCgp39) and gp130-RAPS, dnaJp1, citrullinated proteins, citrullinated peptides, citrullinated type II collagen, citrullinated vimentin, citrullinated fibrinogen, myelin basic protein, proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG), thyroid stimulating factor receptor (TSH-R), acetylcholine receptor (AchR), gliadin, histones, PLP, glucose-6-phosphate isomerase, thyroglobulin, various tRNA synthetases, proteinase-3, human desmoglein 2 (DSG2) and myeloperoxidase (MPO).

[0088] As used herein, the term “citrullinated” refers to a peptide antigen which has been post-translationally modified by deamination or citrullination at an arginine residue at e.g., amino acid position 64 of full length amino acid sequence of Vimentin or amino acid position 24 of SEQ ID NO: 7, amino acid position 64 of full length amino acid sequence of Vimentin or amino acid position 27 of SEQ ID NO: 8, amino acid positions 210, 230 and 235 of full length amino acid sequence of Aggrecan or amino acid positions 11, 32 and 37 of SEQ ID NO:11, amino acid position, 876 and 877 of full length amino acid sequence of Tenascin or amino acid positions 6 and 7 of SEQ ID NO: 56, amino acid positions 1014 and 1016 of full length amino acid sequence of Tenascin or amino acid positions 3 and 5 of SEQ ID NO: 57, amino acid position 1637 of full length amino acid sequence of Tenascin or amino acid position 5 of SEQ ID NO: 58, amino acid positions 2073 and 2077 of full length amino acid sequence of Tenascin or amino acid positions 7 and 11 of SEQ ID NO: 59. For example, as used herein citrullination is denoted in the amino acid sequence by X.

[0089] The autoantigen may be derived from CII. For example, CII may be CII₂₅₉₋₂₇₃. In some examples, CII₂₅₉₋₂₇₃ comprises, consists of or consists essentially of an amino acid sequence set forth in SEQ ID NO: 1, or a fragment, variant or derivative thereof. In certain examples, the variant of CII₂₅₉₋₂₇₃ may comprise an amino acid sequence that demonstrates at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence set forth in SEQ ID NO: 1.

[0090] The autoantigen may be derived from PI. For example, PI may be PI₃₃₋₆₃. In some examples, PI₃₃₋₆₃ comprises, consists of or consists essentially of an amino acid sequence set forth in SEQ ID NO: 2, or a fragment, variant or derivative thereof. In certain examples, the variant of PI₃₃₋₆₃ may comprise an amino acid sequence that demonstrates at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence set forth in SEQ ID NO: 2.

[0091] The autoantigen may be derived from OVA. For example, OVA may be OVA₃₂₃₋₃₃₉. In some examples, OVA₃₂₃₋₃₃₉ comprises, consists of or consists essentially of an amino acid sequence set forth in SEQ ID NO: 3, or a fragment, variant or derivative thereof. In certain examples, the variant of OVA₃₂₃₋₃₃₉ may comprise an amino acid sequence that demonstrates at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence set forth in SEQ ID NO: 3.

[0092] The autoantigen may be derived from aggrecan. For example, the Aggrecan antigen may comprise, consist of or consist essentially of an amino acid sequence set forth in any one of SEQ ID NOs: 4 and 11-24 and 69, or a fragment, variant or derivative thereof. In certain examples, the variant of the Aggrecan may comprise an amino acid sequence that demonstrates at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOs: 4 and 11-24 and 69.

[0093] In one example, Aggrecan may be derived from Aggrecan₈₄₋₁₀₃Cit93. In some examples, Aggrecan₈₄₋₁₀₃Cit93 comprises, consists of or consists essentially of an amino acid sequence set forth in SEQ ID NO: 4, or a fragment, variant or derivative thereof. In certain examples the variant of Aggrecan₈₄₋₁₀₃Cit93 may comprise an amino acid sequence that demonstrates at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence set forth in SEQ ID NO: 4.

[0094] In one example, Aggrecan may be Aggrecan₈₉₋₁₀₃. In some examples, Aggrecan₈₉₋₁₀₃ comprises, consists of or consists essentially of an amino acid sequence set forth in SEQ ID NO: 69, or a fragment, variant or derivative thereof. In certain examples the variant of Aggrecan₈₉₋₁₀₃ may comprise an amino acid sequence that demonstrates at least 80%, 81%, 82%, 83%, 84%, 85%,

86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence set forth in SEQ ID NO: 69.

[0095] The autoantigen may be derived from Ro. The autoantigen may be Ro60. For example, Ro may be Ro60₄₀₁₋₄₂₅. In some examples, Ro60₄₀₁₋₄₂₅ comprises, consists of or consists essentially of an amino acid sequence set forth in SEQ ID NO: 5, or a fragment, variant or derivative thereof. In certain examples, the variant of Ro60₄₀₁₋₄₂₅ may comprise an amino acid sequence that demonstrates at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence set forth in SEQ ID NO: 5.

[0096] The autoantigen may be derived from Vimentin. For example, the Vimentin may comprise, consist of or consist essentially of an amino acid sequence set forth in any one of SEQ ID NOs: 6-8, or a fragment, variant or derivative thereof. In certain examples, the variant of the Vimentin may comprise an amino acid sequence that demonstrates at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOs: 6-8. For example, the Vimentin may comprise, consist of or consist essentially of an amino acid sequence set forth in any one of SEQ ID NOs: 6-8, 66-68 and 70, or a fragment, variant or derivative thereof. In certain examples, the variant of Vimentin may comprise an amino acid sequence that demonstrates at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOs: 6-8, 66-68 and 70. For example, the Vimentin may be derived from an amino acid sequence set forth in SEQ ID NO: 68.

[0097] In one example, Vimentin-derived autoantigen may be Vimentin₄₁₋₈₅. In some examples, Vimentin₄₁₋₈₅ comprises, consists of or consists essentially of an amino acid sequence set forth in SEQ ID NO: 6, or a fragment, variant or derivative thereof. In certain examples, the variant of Vimentin₄₁₋₈₅ may comprise an amino acid sequence that demonstrates at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence set forth in SEQ ID NO: 6.

[0098] In another example, Vimentin-derived autoantigen may be Vimentin₄₁₋₈₅Cit64. In some examples, Vimentin₄₁₋₈₅Cit64 comprises, consists of or consists essentially of an amino acid sequence set forth in SEQ ID NO: 7, or a fragment, variant or derivative thereof. In certain examples, the variant of Vimentin₄₁₋₈₅Cit64 may comprise an amino acid sequence that demonstrates at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence set forth in SEQ ID NO: 7.

[0099] In another example, Vimentin-derived autoantigen may be Vimentin₃₈₋₈₈Cit64. In some examples, Vimentin₃₈₋₈₈Cit64 comprises, consists of or consists essentially of an amino acid sequence set forth in SEQ ID NO: 8, or a fragment, variant or derivative thereof. In certain examples, the variant of Vimentin₃₈₋₈₈Cit64 may comprise an amino acid sequence that demonstrates at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence set forth in SEQ ID NO: 8.

[0100] In another example, Vimentin-derived autoantigen may be Vimentin₅₃₋₈₅Cit64. In some examples, Vimentin₅₃₋₈₅Cit64 comprises, consists of or consists essentially of an amino acid sequence set forth in SEQ ID NO: 66, or a fragment, variant or derivative thereof. In certain examples, the variant of Vimentin₅₃₋₈₅Cit64 may comprise an amino acid sequence that demonstrates at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence set forth in SEQ ID NO: 66.

[0101] In another example, Vimentin-derived autoantigen may be Vimentin₆₀₋₈₅Cit64. In some examples, Vimentin₆₀₋₈₅Cit64 comprises, consists of or consists essentially of an amino acid sequence set forth in SEQ ID NO: 67, or a fragment, variant or derivative thereof. In certain examples, the variant of Vimentin₆₀₋₈₅Cit64 may comprise an amino acid sequence that demonstrates at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence set forth in SEQ ID NO: 67.

[0102] In another example, Vimentin-derived autoantigen may be Vimentin₅₉₋₇₁. In some examples, Vimentin₅₉₋₇₁ comprises, consists of or consists essentially of an amino acid sequence set forth in SEQ ID NO: 70, or a fragment, variant or derivative thereof. In certain examples, the variant of Vimentin₅₉₋₇₁ may comprise an amino acid sequence that demonstrates at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence set forth in SEQ ID NO: 70.

[0103] The autoantigen may be derived from myeloperoxidase (MPO). For example, the MPO may comprise, consist of or consist essentially of an amino acid sequence set forth in any one of SEQ ID NOs: 9 and 10, or a fragment, variant or derivative thereof. In certain examples, the variant of the MPO may comprise an amino acid sequence that demonstrates at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOs: 9 and 10.

[0104] The autoantigen may be derived from thyroid stimulating factor receptor (TSH). For example, the TSH may comprise, consist of or consist essentially of an amino acid sequence set

forth in any one of SEQ ID NOs: 25-55, or a fragment, variant or derivative thereof. In certain examples, the variant of the TSH may comprise an amino acid sequence that demonstrates at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOs: 25-55.

[0105] The autoantigen may be derived from Tenascin C. For example, the Tenascin C may comprise, consist of or consist essentially of an amino acid sequence set forth in any one of SEQ ID NOs: 56-59, or a fragment, variant or derivative thereof. In certain examples, the variant of the Tenascin C may comprise an amino acid sequence that demonstrates at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOs: 56-59.

[0106] The autoantigen may be derived from desmoglein-2. For example, the desmoglein-2 may comprise, consist of or consist essentially of an amino acid sequence set forth in SEQ ID NO: 71, or a fragment, variant or derivative thereof. In certain examples, the variant of the desmoglein-2 may comprise an amino acid sequence that demonstrates at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence set forth in SEQ ID NO: 71.

[0107] A peptide antigen of the present disclosure may be soluble in water. As will be understood by the skilled person, “water solubility” is typically influenced by the amino acid composition of the peptide, including the number of charged, non-charged, polar or non-polar amino acids. Water solubility of a peptide antigen may be determined by methods known in the art, including but not limited to, the gravimetric method, UV-Vis spectroscopy, HPLC, dynamic light scattering (DLS) and a solubility test. A peptide antigen of the present disclosure may be soluble in water as determined by the gravimetric method. A peptide antigen of the present disclosure may be soluble in water as determined by UV-Vis spectroscopy. A peptide antigen of the present disclosure may be soluble in water as determined by HPLC. A peptide antigen of the present disclosure may be soluble in water as determined by dynamic light scattering (DLS). A peptide antigen of the present disclosure may be soluble in water as determined by a solubility test.

[0108] A peptide antigen of the present disclosure may have a negative or neutral charge. A peptide antigen of the present disclosure may have a negative charge. A peptide antigen of the present disclosure may have a neutral charge. The charge of a peptide antigen may be determined by methods known in the art, including but not limited to, calculating the net charge of the peptide antigen. The charge of the peptide antigen may be changed by adjusting the pH of the solution comprising the peptide antigen. For example, to prepare a peptide antigen with a neutral charge the pH of the solution comprising the peptide antigen is adjusted to the isoelectric point (pI) of the

peptide antigen. Alternatively, to prepare a peptide antigen with a negative charge the pH of the solution comprising the peptide antigen is adjusted above the pI of the peptide antigen.

[0109] A peptide antigen of the present disclosure may comprise a plurality of epitopes. For example, a polyepitope peptide antigen may comprise 30 or less (e.g., 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 etc or any range therein) epitopes in total. Alternatively, a peptide antigen may comprise 20 or less (e.g., 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 etc or any range therein) epitopes in total.

[0110] As used herein, the term “epitope” refers to any protein determinant capable of specific binding to an immunoglobulin or fragment thereof. In some examples, the peptide antigens described herein may comprise multiple epitopes. For example, the peptide antigens described herein may be included or contained within a polyepitope protein or a polytope protein. A polyepitope protein may comprise one or a plurality of human leukocyte antigen (HLA) epitopes. More particularly, the polyepitope protein of the present disclosure may include two or more different peptide antigens described herein. The skilled person will appreciate that the particular number and/or type of the constituent epitopes of the peptide antigen and/or the polyepitope protein may readily be altered while retaining broad HLA immunogenicity. In addition to the epitopes, the peptide antigen or the polytope protein may further comprise additional or intervening amino acids or amino acid sequences (e.g., linker sequences). Intervening amino acids or amino acid sequences may be present between at least two of the epitope amino acid sequences, or between each adjacent epitope amino acid sequence. It is further contemplated that additional amino acids or amino acid sequences may be present at one or both of an N-terminal end and a C-terminal end of the peptide antigen or the polyepitope protein.

[0111] It will be appreciated by a skilled person that the epitope or epitopes selected for inclusion in the peptide antigen and/or the polyepitope protein may be tailored to fit any population, race or other group of individuals.

[0112] Other criteria for inclusion of particular epitopes within the peptide antigen include those (i) having minimal or no sequence variants; and (ii) selected from HLAs having minimal subtypes.

[0113] The isolated proteins, including the polyepitope protein described herein, may comprise a plurality of epitopes derived from a plurality of different protein antigens.

[0114] The peptide antigen or the polyepitope protein described herein may have epitopes derived from one or more proteins (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 etc or more proteins), two or more proteins, three or more proteins, four or more proteins or five or more proteins selected from the group comprising Ro (e.g., Ro₄₀₁₋₄₂₅), collagen type II (CII) (e.g., CII₂₅₉₋₂₇), proinsulin (PI) (e.g., PI₃₃₋₆₃), insulin, chromogranin, aggrecan (e.g., aggrecan₂₀₀₋₂₄₄), islet antigen 2 (IA2), glutamic acid decarboxylase 65-kilodalton isoform (GAD65), glycoprotein (gp70), nuclear antigens, lupus

autoantigen, Smith, La, U1-RNP, fibrillin, histones, ribosomal proteins, pyruvate dehydrogenase dihydrolipoamide acetyltransferase (PCD-E2), hair follicle antigens, human tropomyosin isoform 5 (hTM5), human cartilage gp 39 (HCgp39) and gp130-RAPS, dnaJp1, citrullinated proteins, citrullinated peptides, citrullinated type II collagen, citrullinated vimentin (e.g., Vimentin₄₁₋₈₅ Cit64, Vimentin₅₃₋₈₅ Cit64 or Vimentin₆₀₋₈₅ Cit64), citrullinated fibrinogen, citrullinated aggrecan (e.g. Aggrecan₂₀₀₋₂₄₄Cit210,Cit230,Cit235), citrullinated tenascin C (e.g., Tenascin-C₁₀₁₂₋₁₀₂₆Cit1014,Cit1016) myelin basic protein, proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG), thyroid stimulating factor receptor (TSH-R e.g. TSHR₅₇₋₇₅, TSHR₇₀₋₈₈, TSHR₂₂₇₋₂₄₂, TSHR₃₁₅₋₃₃₄), acetylcholine receptor (AchR), gliadin, histones, PLP, glucose-6-phosphate isomerase, thyroglobulin, various tRNA synthetases, proteinase-3 and myeloperoxidase (MPO e.g. MPO₄₃₅₋₄₆₅, MPO₄₄₇₋₄₅₉) and combinations thereof.

[0115] A polyepitope protein may comprise two or more different epitopes derived from two or more different peptide antigens or proteins described herein. For example, a polyepitope protein may comprise an epitope derived from PI and an epitope derived from Vimentin. For example, a polyepitope protein may comprise an epitope derived from type II collagen and an epitope derived from Vimentin. For example, a polyepitope protein may comprise a native epitope derived from PI and a hybrid insulin peptide (HIP) epitope derived from PI. More particularly, the polyepitope protein may comprise a first amino acid sequence derived from an amino acid sequence set forth in SEQ ID NO: 2 (i.e., PI₃₃₋₆₃) and a second amino acid sequence derived from an amino acid sequence set forth in any one of SEQ ID NOs: 60-65. More particularly, the polyepitope protein may comprise a first amino acid sequence that comprises, consists of or consists essentially of an amino acid sequence set forth in SEQ ID NO: 2 (i.e., PI₃₃₋₆₃) and a second amino acid sequence that comprises, consists of or consists essentially of the amino acid sequence set forth in SEQ ID NO: 7 (i.e., Vimentin₄₁₋₈₅Cit64). In another example, the polyepitope protein may comprise a first amino acid sequence derived from an amino acid sequence set forth from SEQ ID NO: 7 and a second amino acid sequence derived from a second or third citrullinated or non-citrullinated RA autoantigen (e.g., any one of SEQ ID NOs: 1, 4, 11-24 and 56-59). In one example, the polyepitope protein may comprise a first amino acid sequence that comprises, consists of or consists essentially of an amino acid sequence set forth in SEQ ID NO: 66 and a second amino acid sequence that comprises, consists of or consists essentially of the amino acid sequence set forth in any one of SEQ ID NOs: 1, 4-6, 11-24 and 56-59.

[0116] As used herein, a “hybrid insulin peptide” or “HIP” will be understood to refer to a type of neoepitope formed by the fusion of two unrelated peptide fragments. HIPs are created by the covalent cross-linking of proinsulin peptides with other peptides present in β cell secretory granules and are recognised by pathogenic CD4 T cells. Delong, T., et al., Science, 2016.

351(6274): p. 711-714 and Tran, M., et al., Journal of Biological Chemistry, Volume 300, Issue 9, 107612 incorporated herein by reference both discuss how pathogenic CD4 T cells in type 1 diabetes recognise HIPs.

[0117] In one example, the HIP comprises, consists of or consists essentially of an amino acid sequence set forth in any one of SEQ ID NOs: 60-65, or a fragment, variant or derivative thereof. In certain examples, the variant thereof may comprise an amino acid sequence that demonstrates at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOs: 60-65.

[0118] In one example, the HIP comprises, consists of or consists essentially of an amino acid sequence set forth in SEQ ID NO: 60, or a fragment, variant or derivative thereof. In certain examples, the variant thereof may comprise an amino acid sequence that demonstrates at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOs: 60.

[0119] In one example, the HIP comprises, consists of or consists essentially of an amino acid sequence set forth in SEQ ID NO: 61, or a fragment, variant or derivative thereof. In certain examples, the variant thereof may comprise an amino acid sequence that demonstrates at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOs: 61.

[0120] In one example, the HIP comprises, consists of or consists essentially of an amino acid sequence set forth in SEQ ID NO: 62, or a fragment, variant or derivative thereof. In certain examples, the variant thereof may comprise an amino acid sequence that demonstrates at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOs: 62.

[0121] In one example, the HIP comprises, consists of or consists essentially of an amino acid sequence set forth in SEQ ID NO: 63, or a fragment, variant or derivative thereof. In certain examples, the variant thereof may comprise an amino acid sequence that demonstrates at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOs: 63.

[0122] In one example, the HIP comprises, consists of or consists essentially of an amino acid sequence set forth in SEQ ID NO: 64, or a fragment, variant or derivative thereof. In certain

examples, the variant thereof may comprise an amino acid sequence that demonstrates at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOs: 64.

[0123] In one example, the HIP comprises, consists of or consists essentially of an amino acid sequence set forth in SEQ ID NO: 65, or a fragment, variant or derivative thereof. In certain examples, the variant thereof may comprise an amino acid sequence that demonstrates at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOs: 65.

[0124] Alternatively, a polypeptide protein may comprise two or more copies of a single epitope derived from a single peptide antigen described herein. In other examples, the polypeptide protein comprises two or more different epitopes derived from a single peptide antigen described herein.

[0125] Contemplated herein are “variant” peptide antigens. As used herein, a nucleic acid “variant” shares a definable amino acid sequence relationship with a reference amino acid sequence (e.g., any one of SEQ ID NOs: 1-71). The “variant” amino acid may have one or a plurality of amino acids of the reference amino acid sequence deleted, modified, conjugated and/or substituted by different amino acids. The “variant” amino acid sequence may be truncated or elongated, while still retaining a definable amino acid sequence relationship with a reference amino acid sequence over a contiguous stretch of amino acids. It is well understood in the art that conservative amino acids of a protein may be substituted or deleted without changing (or only having minimal change to) the activity of the protein. Suitably, amino acid variants share at least 60% or 65%, 66%, 67%, 68%, 69%, preferably at least 70%, 71%, 72%, 73%, 74% or 75%, more particularly at least 80%, 81%, 82%, 83%, 84%, or 85%, and even more particularly at least 90%, 91%, 92%, 93%, 94%, or 95% amino acid sequence identity with an amino acid of the disclosure (e.g., SEQ ID NOs: 1-71). Percent sequence identity may be determined by any method known in the art, such as that described herein.

[0126] Terms used generally herein to describe sequence relationships between respective amino acids and nucleic acids include “comparison window”, “sequence identity”, “percentage of sequence identity” and “substantial identity”. Because respective nucleic acids/proteins may each comprise (1) only one or more portions of a complete nucleic acid/ amino acid sequence that are shared by the nucleic acids/ amino acids, and (2) one or more portions which are divergent between the nucleic acids/ amino acids, sequence comparisons are typically performed by comparing sequences over a “comparison window” to identify and compare local regions of sequence similarity. A “comparison window” refers to a conceptual segment of typically 6, 9 or 12

contiguous residues that is compared to a reference sequence. The comparison window may comprise additions or deletions (i.e., gaps) of about 20% or less as compared to the reference sequence for optimal alignment of the respective sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by computerised implementations of algorithms (Geneworks program by Intelligenetics; GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Drive Madison, WI, USA, incorporated herein by reference) or by inspection and the best alignment (i.e., resulting in the highest percentage homology over the comparison window) generated by any of the various methods selected. Reference also may be made to the BLAST family of programs as for example disclosed by Altschul et al., 1997, Nucl. Acids Res. 25 3389, which is incorporated herein by reference. A detailed discussion of sequence analysis can be found in Unit 19.3 of CURRENT PROTOCOLS IN MOLECULAR BIOLOGY Eds. Ausubel et al. (John Wiley & Sons Inc NY, 1995-1999).

[0127] The term “sequence identity” is used herein in its broadest sense to include the number of exact nucleotide or amino acid matches having regard to an appropriate alignment using a standard algorithm, having regard to the extent that sequences are identical over a window of comparison. Thus, a “percentage of sequence identity” is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G, U) or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity. For example, “sequence identity” may be understood to mean the “match percentage” calculated by the DNASIS computer program (Version 2.5 for windows; available from Hitachi Software engineering Co., Ltd., South San Francisco, California, USA).

[0128] Also contemplated herein are amino acid fragments, such as peptide antigen fragments. A “fragment” is a segment, domain, portion or region of an amino acid, which respectively constitutes less than 100% of the amino acid sequence. In particular examples, an amino acid fragment may comprise, for example, at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 55, 60, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79 (inclusive of any range therein) contiguous amino acids of said amino acid sequence (e.g., SEQ ID NOs: 1-71).

[0129] Further contemplated herein are nucleic acid derivatives, inclusive of peptide antigen derivatives. Such peptide antigen derivatives may include, for example, one or more modifications and/or conjugates as described herein or known in the art.

[0130] An “alloantigen” shall be understood to mean an antigen found only in some members of a species, such as blood group antigens. Examples of alloantigens may include, but are not limited to, glycoproteins (e.g., MHC, MNS antigens), sialoglycoproteins (e.g., CD43), oligosaccharides (e.g., ABO(H), Secretor, Lewis, Li and P), sialo-oligosaccharides (e.g., sialyl-Lewis^x or sialyl-Lewis^a) and proteins (e.g., Rhesus).

[0131] Examples of allergens may include, but are not limited to, Fel d 1 (i.e., the feline skin and salivary gland allergen of the domestic cat *Pelis domesticus*, the amino acid sequence of which is described WO1991006571A1 and incorporated herein by reference), Der p I, Der p II, Der fI or Der fII (i.e., the major protein allergens from the house dust mite dermatophagoides, the amino acid sequence of which is described in WO1994024281A1 and incorporated herein by reference).

[0132] Allergens may also be derived, for example, from any one of the following: grass, tree and weed (including ragweed) pollens; fungi and moulds; foods such as fish, shellfish, crab, lobster, peanuts, nuts, wheat gluten, eggs and milk; stinging insects such as bee, wasp, and hornet and the chironomidae (non-biting midges); other insects such as the housefly, fruit fly, sheep blow fly, screw worm fly, grain weevil, silkworm, honeybee, non-biting midge larvae, bee moth larvae, mealworm, cockroach and larvae of *Tenebrio molitor* beetle; spiders and mites, including the house dust mite; allergens found in the dander, urine, saliva, blood or other bodily fluid of mammals such as cat, dog, cow, pig, sheep, horse, rabbit, rat, guinea pig, mouse and gerbil; airborne particulates in general; latex; and protein detergent additives.

[0133] The peptide antigen may be isolated from a natural source or may be prepared by recombinant techniques as is known in the art. For example, the peptide antigen can be eluted from the major histocompatibility complex (MHC) and other presenting molecules of antigen-presenting cells obtained from a cell population or tissue for which a modified immune response is desired, e.g., an allogeneic tissue or cell population in transplantation medicine. The eluted peptides can be purified using standard protein purification techniques known in the art (Rawson et al., 2000, Cancer Res 60(16), 4493-4498). If desired, the purified peptides can be sequenced and synthetic versions of the peptides produced using standard protein synthesis techniques as for example described below.

[0134] Alternatively, crude antigen preparations can be produced by isolating a sample of a cell population or tissue for which a modified immune response is desired, and either lysing the sample or subjecting the sample to conditions that will lead to the formation of apoptotic cells (e.g., irradiation with ultra violet or with gamma rays, viral infection, cytokines or by depriving cells of nutrients in the cell culture medium, incubation with hydrogen peroxide, or with drugs such as dexamethasone, ceramide chemotherapeutics and anti-hormonal agents such as Lupron or

Tamoxifen). The lysate or the apoptotic cells can then be used as a source of crude antigen for contact with the antigen presenting cells.

[0135] A peptide antigen may be conveniently prepared in recombinant form using standard protocols as for example described in: Sambrook, et al., *Molecular Cloning. A Laboratory Manual* (Cold Spring Harbor Press, 1989), in particular Sections 16 and 17; Ausubel et al., *Current Protocols in Molecular Biology* (John Wiley & Sons, Inc. 1994-1998), in particular Chapters 10 and 16; and Coligan et al., *Current Protocols In Protein Science* (John Wiley & Sons, Inc. 1995-1997), in particular, Chapters 1, 5 and 6.

[0136] A peptide antigen may be prepared by a procedure including the steps of: (a) providing an expression vector from which the peptide antigen or analogue or mimetic thereof is expressible; (b) introducing the vector into a suitable host cell; (c) culturing the host cell to express recombinant polypeptide from the vector; and (d) isolating the recombinant polypeptide.

[0137] Alternatively, the peptide antigen can be synthesised using solution synthesis or solid phase synthesis as described, for example, by Atherton and Sheppard (*Solid 15 Phase Peptide Synthesis: A Practical Approach*, IRL Press at Oxford University Press, Oxford, England, 1989) or by Roberge et al. (1995, *Science* 269: 202).

[0138] A peptide antigen may be present in the lipid delivery vehicle described herein in an amount of about 1 µg/mL to about 50 µg/mL. For example, a peptide antigen may be present in the lipid delivery vehicle described herein in an amount of about 10 µg/mL to about 30 µg/mL. For example, a peptide antigen may be present in an amount of about 1 µg/mL, or about 2 µg/mL, or about 3 µg/mL, or about 4 µg/mL, or about 5 µg/mL, or about 6 µg/mL, or about 7 µg/mL, or about 8 µg/mL, or about 9 µg/mL, or about 10 µg/mL, or about 11 µg/mL, or about 12 µg/mL, or about 13 µg/mL, or about 14 µg/mL, or about 15 µg/mL, or about 16 µg/mL, or about 17 µg/mL, or about 18 µg/mL, or about 19 µg/mL, or about 20 µg/mL, or about 21 µg/mL, or about 22 µg/mL, or about 23 µg/mL, or about 24 µg/mL, or about 25 µg/mL, or about 6 µg/mL, or about 27 µg/mL, or about 28 µg/mL, or about 29 µg/mL, or about 30 µg/mL, or about 31 µg/mL, or about 32 µg/mL, or about 33 µg/mL, or about 34 µg/mL, or about 35 µg/mL, or about 36 µg/mL, or about 37 µg/mL, or about 38 µg/mL, or about 39 µg/mL, or about 40 µg/mL, or about 41 µg/mL, or about 42 µg/mL, or about 43 µg/mL, or about 44 µg/mL, or about 45 µg/mL, or about 46 µg/mL, or about 47 µg/mL, or about 48 µg/mL, or about 49 µg/mL, or about 50 µg/mL.

[0139] For example, an autoantigen derived from proinsulin (e.g., SEQ ID NO: 2) may be present in the lipid delivery vehicle described herein in an amount of about 1 µg/mL to about 50 µg/mL. For example, an autoantigen derived from proinsulin (e.g., SEQ ID NO: 2) may be present in the lipid delivery vehicle described herein in an amount of about 10 µg/mL to about 30 µg/mL. For example, an autoantigen derived from proinsulin (e.g., SEQ ID NO: 2) may be present in an

amount of about 1 µg/mL, or about 2 µg/mL, or about 3 µg/mL, or about 4 µg/mL, or about 5 µg/mL, or about 6 µg/mL, or about 7 µg/mL, or about 8 µg/mL, or about 9 µg/mL, or about 10 µg/mL, or about 11 µg/mL, or about 12 µg/mL, or about 13 µg/mL, or about 14 µg/mL, or about 15 µg/mL, or about 16 µg/mL, or about 17 µg/mL, or about 18 µg/mL, or about 19 µg/mL, or about 20 µg/mL, or about 21 µg/mL, or about 22 µg/mL, or about 23 µg/mL, or about 24 µg/mL, or about 25 µg/mL, or about 26 µg/mL, or about 27 µg/mL, or about 28 µg/mL, or about 29 µg/mL, or about 30 µg/mL, or about 31 µg/mL, or about 32 µg/mL, or about 33 µg/mL, or about 34 µg/mL, or about 35 µg/mL, or about 36 µg/mL, or about 37 µg/mL, or about 38 µg/mL, or about 39 µg/mL, or about 40 µg/mL, or about 41 µg/mL, or about 42 µg/mL, or about 43 µg/mL, or about 44 µg/mL, or about 45 µg/mL, or about 46 µg/mL, or about 47 µg/mL, or about 48 µg/mL, or about 49 µg/mL, or about 50 µg/mL.

[0140] A surprising feature of the disclosed lipid delivery vehicles comprising 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG) is the ability of the lipid delivery vehicle to retain the encapsulated peptide and/or the nuclear factor-κB (NF-κB) inhibitor within the lipid delivery vehicle. Retention of the encapsulated peptide and/or the nuclear factor-κB (NF-κB) inhibitor within the lipid delivery vehicle is surprising in view of lipid delivery vehicles being notoriously “leaky”, in that APIs are typically not retained well, and said leakiness is exacerbated by time, making storage stability an issue, as well as by incubation in biological fluids, maying targeted subcutaneous delivery less effective. Thus, the presently disclosed lipid delivery vehicles are advantageous for the purposes of storage of an aqueous formulation/composition comprising loaded lipid delivery vehicles, and particularly advantageous for an aqueous formulation/composition comprising loaded lipid delivery vehicles that is intended for subcutaneous delivery, as these must retain the encapsulated peptide and/or the nuclear factor-κB (NF-κB) inhibitor within the lipid delivery vehicle during circulation in the recipient plasma.

[0141] As used herein, “retention” of the peptide antigen and/or NF-κB inhibitor refers to encapsulation stability or efficiency of the APIs (e.g., the peptide antigen and/or NF-κB inhibitor) *in vitro* or *in vivo* and may be measured using any methods known in the art, including those described or developed herein (e.g., concentration after storage at 4°C or -20°C *in vitro*, or incubation at 37°C with PBS or plasma followed by SEC to estimate *in vivo* stability in plasma, and/or a peptidase assay).

[0142] The liposomal concentration of the peptide antigen and/or NF-κB inhibitor may be measured using any methods known in the art, including those described herein (e.g., mass spectrometry, enzyme-linked immunosorbent assay (ELISA)).

[0143] Retention of the peptide antigen may be measured prior to and/or after incubation with plasma using mass spectrometry (e.g., as described in the Examples). Retention of the peptide antigen may be calculated by measuring retention of the peptide antigen prior to incubation with plasma using mass spectrometry and measuring retention of the peptide antigen after incubation with plasma using mass spectrometry and determining the difference in the amount of peptide antigen.

[0144] Retention of the NF- κ B inhibitor may be measured prior to and/or after incubation with plasma using ELISA (e.g., as described in the Examples). Retention of the NF- κ B inhibitor may be calculated by measuring retention of the NF- κ B inhibitor prior to incubation with plasma using ELISA and measuring retention of the NF- κ B inhibitor after incubation with plasma using ELISA and determining the difference in the amount of NF- κ B inhibitor.

[0145] When expressed as a percentage, a percentage retention of the peptide antigen and/or NF- κ B inhibitor refers to the liposomal concentration of the peptide antigen and/or NF- κ B inhibitor after incubation for a certain period of time at a particular set of conditions (i.e. for 15 minutes, at 37°C, in plasma; or for 1 hour, at 20°C in PBS), as a percentage of the liposomal concentration of the peptide antigen and/or NF- κ B inhibitor prior to the incubation. This may also be expressed as a percentage of the peptide antigen and/or NF- κ B inhibitor retained within the lipid delivery vehicle.

[0146] The retention of the peptide antigen within the lipid delivery vehicle may be between 25% to 100%. The retention of the peptide antigen within the lipid delivery vehicle may be between 50% to 100%. The retention of the peptide antigen within the lipid delivery vehicle may be between 70% to 100%. The retention of the peptide antigen within the lipid delivery vehicle may be between 70% to 90%. The retention of the peptide antigen within the lipid delivery vehicle may be at least 25%, for example about 25%, or 30%, or 35%, or 40%, or 45%, or 50%, or 55%, or 60%, or 65%, or 70%, or 75%, or 80%, or 85%, or 90%, or 91%, or 92%, or 93%, or 94%, or 95%, or 96%, or 97%, or 98%, or 99%, or 100%. In some examples, the retention may be at least 40%. In certain examples, the retention may be at least 50%.

NF- κ B inhibitors

[0147] As used herein, the term “nuclear factor- κ B” or “NF- κ B” shall be understood to refer to a protein complex that controls transcription of DNA (e.g., an immunomodulator). NF- κ B is found in most cell types and is involved in cellular responses to stimuli such as stress, cytokines, free radicals, ultraviolet irradiation, oxidized LDL, and bacterial or viral antigens. NF- κ B plays a key role in regulating the immune response to infection and incorrect regulation of NF- κ B has been

linked to various diseases. The NF- κ B signaling pathway and components thereof are known in the art.

[0148] The term “nuclear factor- κ B inhibitor” or “NF- κ B inhibitor” shall be understood to refer to any molecule or compound that increases or decreases the level or functional activity of NF- κ B signaling or proteins of the NF- κ B signaling pathway. For example, the NF- κ B inhibitors may be inhibitors that have been determined to inhibit the activity of an NF- κ B protein. Alternatively, the NF- κ B inhibitor may be a modulator of a protein of the pathway that inhibits active NF- κ B signaling.

[0149] As used herein, the term “decrease” shall be understood to mean that the level and/or functional activity of a member of the NF- κ B signaling pathway or a protein of the NF- κ B signaling pathway is reduced relative to the naturally occurring level and/or functional activity of a member of the NF- κ B signaling pathway or a protein of the NF- κ B signaling pathway. For example, a “decrease” in the level and/or functional activity of a member of the NF- κ B signaling pathway or a protein of the NF- κ B signaling pathway is reduced relative to the naturally occurring level and/or functional activity of a member of the NF- κ B signaling pathway or a protein of the NF- κ B signaling pathway prior to administration of the NF- κ B inhibitor.

[0150] As used herein, the term “increase” shall be understood to mean that the level and/or functional activity of a member of the NF- κ B signaling pathway or a protein of the NF- κ B signaling pathway is elevated relative to the naturally occurring level and/or functional activity of a member of the NF- κ B signaling pathway or a protein of the NF- κ B signaling pathway. For example, an “increase” in the level and/or functional activity of a member of the NF- κ B signaling pathway or a protein of the NF- κ B signaling pathway is elevated relative to the naturally occurring level and/or functional activity of a member of the NF- κ B signaling pathway or a protein of the NF- κ B signaling pathway prior to administration of the NF- κ B inhibitor.

[0151] The NF- κ B inhibitor may decrease the level and/or functional activity of a member of the NF- κ B signaling pathway selected from the group consisting of BTK, LYN, BCR Ig α , BCR Ig β , Syk, Btk, PLC γ 2, PKC β , DAG, CARMA1, BCL10, MALT1, PI3K, PIP3, AKT, p38 MAPK, ERK, COT, IKK α , IKK β , IKK γ , NIK, RelA/p65, P105/p50, cRel, RelB, p52, NIK, Leu13, CD81, CD19, CD21 and its ligands in the complement and coagulation cascade, TRAF6, ubiquitin ligase, Tab2, TAK1, NEMO, NOD2, RIP2, Lek, fyn, Zap70, LAT, GRB2, SOS, CD3 zeta, Slp-76, GADS, ITK, PLC γ 1, PKC θ , ICOS, CD28, SHP2, SAP, SLAM and 2B4 and combinations thereof.

[0152] The NF- κ B inhibitor may decrease the level and/or functional activity of a NF- κ B family protein selected from the group consisting of Rel-A (p65), Rel-B, Rel (c-Rel), NF- κ B1 (p50/p105), and NF- κ B2 (p52/p100) and combinations thereof.

[0153] Suitably, the NF- κ B inhibitor blocks, inhibits or otherwise antagonises at least one function or activity of the member of the NF- κ B signaling pathway.

[0154] The NF- κ B inhibitor may increase the level and/or functional activity of a member of the NF- κ B signaling pathway selected from the group consisting of SHP1, SHIP, PIR-B, CD22, CD72, Fc γ RIIB, I κ B, P100, CTLA4, PD-1, Chi, KIR3DL1, KIR3DL2, KIR2DL and Csk and combinations thereof.

[0155] Suitably, the NF- κ B inhibitor increases, stimulates or otherwise agonises at least one function or activity of the member of the NF- κ B signaling pathway.

[0156] The NF- κ B inhibitor may be a Vitamin D, an active metabolite of Vitamin D, a previtamin D, or a synthetic Vitamin D analog. Vitamin D and related molecules, be they naturally occurring or synthetic, are known to be NF- κ B inhibitors, as well as being useful for a range of other therapeutic applications. However, naturally occurring Vitamin D is not always appropriate for the numerous indications for which Vitamin D has been investigated and/or implicated, and this has led to the discovering and development of a large suite of Vitamin D substitutes, namely, molecules that serve as pre- or pro- versions of Vitamin D, and Vitamin analogs with superior drugability profiles. As this suite of “substitutes” will only increase over time, it will be understood that the present disclosure contemplates the inclusion of any present, and future, discovered or synthesised Vitamin Ds, active metabolites of Vitamin D, previtamin Ds, or synthetic Vitamin D analogs, that have similar or even superior properties to the herein exemplified Vitamin D analog, Calcitriol.

[0157] The NF- κ B inhibitor may be selected from the group consisting of calcitriol, calciferol, ergocalciferol, alfalcidol, doxercalciferol, calcidiol, 22-oxacalcitriol, paricalcitol, tacalcitol, falecalcitriol, eldecalcitol, inecalcitol, seocalcitol, calcipotriene, maxacalcitol, calcifediol, calcipotriol; synthetic analogs thereof and/or side-chain analogs thereof; and combinations thereof.

[0158] The NF- κ B inhibitor may be selected from the group consisting of calcitriol, rapamycin, leflunomide, teriflunomide, bevacizumab, bithionol, bortezomib, doxorubicin hydrochloride, cantharidin, carfilzomib, chromomycin daunorubicinum, digitoxin, ectinascidin, emetine, erlotinib hydrochloride, gemcitabine, irinotecan, quinacrine dihydrochloride, fluorosalan, manidipine hydrochloride, narasin, lestaurtinib, ouabain, pemetrexed disodium, sorafenib tosylate, sunitinib malate, tioconazole, topotecan, tribromsalan, triclabendazolum, zafirlukast, withaferin A, quecertin, curcumin, BAY 11-7085, Bay 11-7082, dexamethasone and combinations thereof. For example, the NF- κ B inhibitor may be calcitriol. Alternatively, the NF- κ B inhibitor may be rapamycin. The NF- κ B inhibitor may be leflunomide. The NF- κ B inhibitor may be teriflunomide. The NF- κ B inhibitor may be bevacizumab. The NF- κ B inhibitor may be bithionol. The NF- κ B inhibitor may be bortezomib. The NF- κ B inhibitor may be doxorubicin hydrochloride. The NF- κ B inhibitor may be cantharidin. The NF- κ B inhibitor may be carfilzomib. The NF- κ B inhibitor may

be chromomycin daunorubicinum. The NF- κ B inhibitor may be digitoxin. The NF- κ B inhibitor may be ectinascidin. The NF- κ B inhibitor may be emetine. The NF- κ B inhibitor may be erlotinib hydrochloride. The NF- κ B inhibitor may be gemcitabine. The NF- κ B inhibitor may be irinotecan. The NF- κ B inhibitor may be quinacrine dihydrochloride. The NF- κ B inhibitor may be fluorosalan. The NF- κ B inhibitor may be manidipine hydrochloride. The NF- κ B inhibitor may be narasin. The NF- κ B inhibitor may be lestaurtinib. The NF- κ B inhibitor may be ouabain. The NF- κ B inhibitor may be pemetrexed disodium. The NF- κ B inhibitor may be sorafenib tosylate. The NF- κ B inhibitor may be sunitinib malate. The NF- κ B inhibitor may be tioconazole. The NF- κ B inhibitor may be topotecan. The NF- κ B inhibitor may be tribromsalan. The NF- κ B inhibitor may be triclabendazolum. The NF- κ B inhibitor may be zafirlukast. The NF- κ B inhibitor may be withaferin A. The NF- κ B inhibitor may be quecertain. The NF- κ B inhibitor may be curcumin. The NF- κ B inhibitor may be BAY 11-7085. The NF- κ B inhibitor may be Bay 11-7082. The NF- κ B inhibitor may be dexamethasone.

[0159] The NF- κ B inhibitor may be non-toxic to a subject. For example, the NF- κ B inhibitor may be produce minimal or negligible side effects in the subject. The NF- κ B inhibitor may block an alternative NF- κ B pathway.

[0160] A NF- κ B inhibitor may be present in the lipid delivery vehicle described herein in an amount of about 10 ng/mL to about 600 ng/mL. Alternatively, a NF- κ B inhibitor may be present in the lipid delivery vehicle described herein in an amount of about 10 ng/mL to about 100 ng/mL. In another alternative, a NF- κ B inhibitor may be present in the lipid delivery vehicle described herein in an amount of about 300 ng/mL to about 600 ng/mL. Alternatively, NF- κ B inhibitor may be present in the lipid delivery vehicle described herein in an amount of about 100 ng/mL to about 300 ng/mL. For example, a NF- κ B inhibitor may be present in an amount of about 10 ng/mL, or about 20 ng/mL, or about 30 ng/mL, or about 40 ng/mL, or about 50 ng/mL, or about 60 ng/mL, or about 70 ng/mL, or about 80 ng/mL, or about 90 ng/mL, or about 100 ng/mL, or about 110 ng/mL, or about 120 ng/mL, or about 130 ng/mL, or about 140 ng/mL, or about 150 ng/mL, or about 160 ng/mL, or about 170 ng/mL, or about 180 ng/mL, or about 190 ng/mL, or about 200 ng/mL, or about 210 ng/mL, or about 220 ng/mL, or about 230 ng/mL, or about 240 ng/mL, or about 250 ng/mL, or about 260 ng/mL, or about 270 ng/mL, or about 280 ng/mL, or about 290 ng/mL, or about 300 ng/mL, or about 310 ng/mL, or about 320 ng/mL, or about 330 ng/mL, or about 340 ng/mL, or about 350 ng/mL, or about 360 ng/mL, or about 370 ng/mL, or about 380 ng/mL, or about 390 ng/mL, or about 400 ng/mL, or about 410 ng/mL, or about 420 ng/mL, or about 430 ng/mL, or about 440 ng/mL, or about 450 ng/mL, or about 460 ng/mL ng/mL, or about 470 ng/mL, or about 480 ng/mL, or about 490 ng/mL, or about 500 ng/mL, or about 510 ng/mL,

or about 520 ng/mL, or about 530 ng/mL, or about 540 ng/mL, or about 550 ng/mL, or about 560 ng/mL, or about 570 ng/mL, or about 580 ng/mL, or about 590 ng/mL, or about 600 ng/mL.

[0161] Calcitriol may be present in an amount of about 100 ng/mL to about 600 ng/mL. Alternatively, Calcitriol may be present in an amount of about 300 ng/mL to about 600 ng/mL. For example, calcitriol may be present in an amount of about 100 ng/mL, or about 110 ng/mL, or about 120 ng/mL, or about 130 ng/mL, or about 140 ng/mL, or about 150 ng/mL, or about 160 ng/mL, or about 170 ng/mL, or about 180 ng/mL, or about 190 ng/mL, or about 200 ng/mL, or about 210 ng/mL, or about 220 ng/mL, or about 230 ng/mL, or about 240 ng/mL, or about 250 ng/mL, or about 260 ng/mL, or about 270 ng/mL, or about 280 ng/mL, or about 290 ng/mL, or about 300 ng/mL, or about 310 ng/mL, or about 320 ng/mL, or about 330 ng/mL, or about 340 ng/mL, or about 350 ng/mL, or about 360 ng/mL, or about 370 ng/mL, or about 380 ng/mL, or about 390 ng/mL, or about 400 ng/mL, or about 410 ng/mL, or about 420 ng/mL, or about 430 ng/mL, or about 440 ng/mL, or about 450 ng/mL, or about 460 ng/mL, or about 470 ng/mL, or about 480 ng/mL, or about 490 ng/mL, or about 500 ng/mL, or about 510 ng/mL, or about 520 ng/mL, or about 530 ng/mL, or about 540 ng/mL, or about 550 ng/mL, or about 560 ng/mL, or about 570 ng/mL, or about 580 ng/mL, or about 590 ng/mL, or about 600 ng/mL.

[0162] The retention of the NF- κ B inhibitor within the lipid delivery vehicle may be between 25% to 100%. The retention of the NF- κ B inhibitor within the lipid delivery vehicle may be between 50% to 100%. The retention of the NF- κ B inhibitor within the lipid delivery vehicle may be between 70% to 100%. The retention of the NF- κ B inhibitor within the lipid delivery vehicle may be between 70% to 90%. The retention of the NF- κ B inhibitor within the lipid delivery vehicle may be at least 25%, for example about 20%, or 25%, or 30%, or 35%, or 40%, or 45%, or 50%, or 60%, or 70%, or 80%, or 90%, or 95%, or 96%, or 97%, or 98%, or 99%, or 100%. In some examples, the retention may be at least 95%. In certain examples, the retention may be at least 99%.

[0163] The lipid delivery vehicle may comprise a NF- κ B inhibitor selected from the group consisting of calcitriol, calciferol, ergocalciferol, alfacalcidol, doxercalciferol, calcidiol, 22-oxacalcitriol, paricalcitol, tacalcitol, falecalcitriol, eldecalcitol, inecalcitol, seocalcitol, calcipotriene, maxacalcitol, calcifediol, calcipotriol; synthetic analogs thereof and/or side-chain analogs thereof; and combinations thereof, and a peptide antigen, wherein the lipid delivery vehicle comprises POPC, POPG, and a PEGylated lipid.

[0164] The lipid delivery vehicle may comprise a NF- κ B inhibitor selected from the group consisting of calcitriol, rapamycin, leflunomide, teriflunomide, bevacizumab, bithionol, bortezomib, doxorubicin hydrochloride, cantharidin, carfilzomib, chromomycin daunorubicinum, digitoxin, ectinascidin, emetine, erlotinib hydrochloride, gemcitabine, irinotecan, quinacrine

dihydrochloride, fluorosalan, manidipine hydrochloride, narasin, lestaurtinib, ouabain, pemetrexed disodium, sorafenib tosylate, sunitinib malate, tioconazole, topotecan, tribromsalan, triclabendazolum, zafirlukast, withaferin A, quecertin, curcumin, BAY 11-7085, Bay 11-7082, dexamethasone and combinations thereof and a peptide antigen, wherein the lipid delivery vehicle comprises POPC and POPG. The lipid delivery vehicle may further comprise a PEGylated lipid.

[0165] The lipid delivery vehicle may comprise a NF- κ B inhibitor selected from the group consisting of calcitriol, calciferol, ergocalciferol, alfacalcidol, doxercalciferol, calcidiol, 22-oxacalcitriol, paricalcitol, tacalcitol, falecalcitriol, eldecalcitol, inecalcitol, seocalcitol, calcipotriene, maxacalcitol, calcifediol, calcipotriol; synthetic analogs thereof and/or side-chain analogs thereof; and combinations thereof, and an autoantigen derived from CII, wherein the lipid delivery vehicle comprises POPC, POPG, a PEGylated lipid. For example, the autoantigen derived from CII may be CII₂₅₉₋₂₇₃ antigen (e.g., SEQ ID NO: 1).

[0166] The lipid delivery vehicle may comprise a NF- κ B inhibitor selected from the group consisting of calcitriol, calciferol, ergocalciferol, alfacalcidol, doxercalciferol, calcidiol, 22-oxacalcitriol, paricalcitol, tacalcitol, falecalcitriol, eldecalcitol, inecalcitol, seocalcitol, calcipotriene, maxacalcitol, calcifediol, calcipotriol; synthetic analogs thereof and/or side-chain analogs thereof; and combinations thereof, and an autoantigen derived from PI, wherein the lipid delivery vehicle comprises POPC, POPG, a PEGylated lipid. For example, the autoantigen derived from PI may be a PI₃₃₋₆₃ antigen (e.g., SEQ ID NO: 2).

[0167] The lipid delivery vehicle may comprise a NF- κ B inhibitor selected from the group consisting of calcitriol, calciferol, ergocalciferol, alfacalcidol, doxercalciferol, calcidiol, 22-oxacalcitriol, paricalcitol, tacalcitol, falecalcitriol, eldecalcitol, inecalcitol, seocalcitol, calcipotriene, maxacalcitol, calcifediol, calcipotriol; synthetic analogs thereof and/or side-chain analogs thereof; and combinations thereof, and an autoantigen derived from AggreCAN, wherein the lipid delivery vehicle comprises POPC, POPG, a PEGylated lipid. For example, the autoantigen derived from AggreCAN may be an AggreCAN₈₄₋₁₀₃Cit93 antigen (e.g., SEQ ID NO: 4). For example, the autoantigen derived from AggreCAN may be an AggreCAN₈₉₋₁₀₃Cit93 antigen (e.g., SEQ ID NO: 69).

[0168] The lipid delivery vehicle may comprise a NF- κ B inhibitor selected from the group consisting of calcitriol, calciferol, ergocalciferol, alfacalcidol, doxercalciferol, calcidiol, 22-oxacalcitriol, paricalcitol, tacalcitol, falecalcitriol, eldecalcitol, inecalcitol, seocalcitol, calcipotriene, maxacalcitol, calcifediol, calcipotriol; synthetic analogs thereof and/or side-chain analogs thereof; and combinations thereof, and an autoantigen derived from AggreCAN, wherein the lipid delivery vehicle comprises POPC, POPG, a PEGylated lipid. For example, the

autoantigen derived from AggreCAN may comprise, consist of or consist essentially of an amino acid sequence set forth in any one of SEQ ID NOs 11-24.

[0169] The lipid delivery vehicle may comprise a NF- κ B inhibitor selected from the group consisting of calcitriol, calciferol, ergocalciferol, alfacalcidol, doxercalciferol, calcidiol, 22-oxacalcitriol, paricalcitol, tacalcitol, falecalcitriol, eldecacitol, inecalcitol, seocalcitol, calcipotriene, maxacalcitol, calcifediol, calcipotriol; synthetic analogs thereof and/or side-chain analogs thereof; and combinations thereof, and an autoantigen derived from Ro, wherein the lipid delivery vehicle comprises POPC, POPG, a PEGylated lipid. For example, the autoantigen derived from Ro may be a Ro₄₀₁₋₄₂₅ antigen (e.g., SEQ ID NO: 5).

[0170] The lipid delivery vehicle may comprise a NF- κ B inhibitor selected from the group consisting of calcitriol, calciferol, ergocalciferol, alfacalcidol, doxercalciferol, calcidiol, 22-oxacalcitriol, paricalcitol, tacalcitol, falecalcitriol, eldecacitol, inecalcitol, seocalcitol, calcipotriene, maxacalcitol, calcifediol, calcipotriol; synthetic analogs thereof and/or side-chain analogs thereof; and combinations thereof, and an autoantigen derived from Vimentin, wherein the lipid delivery vehicle comprises POPC, POPG, a PEGylated lipid. For example, the autoantigen derived from Vimentin may be an Vimentin₄₁₋₈₅ antigen (e.g., SEQ ID NO: 6). In another example, the autoantigen derived from Vimentin may be a Vimentin₄₁₋₈₅Cit64 antigen (e.g., SEQ ID NO: 7). In another example, the autoantigen derived from Vimentin may be a Vimentin₃₈₋₈₈Cit64 antigen (SEQ ID NO: 8). In another example, the autoantigen derived from Vimentin may be a Vimentin₅₃₋₈₅Cit64 antigen (SEQ ID NO: 66). In another example, the autoantigen derived from Vimentin may be a Vimentin₆₀₋₈₅Cit64 antigen (SEQ ID NO: 67). In another example, the autoantigen derived from Vimentin may be a Vimentin₅₉₋₇₁ antigen (SEQ ID NO: 71).

[0171] The lipid delivery vehicle may comprise a NF- κ B inhibitor selected from the group consisting of calcitriol, calciferol, ergocalciferol, alfacalcidol, doxercalciferol, calcidiol, 22-oxacalcitriol, paricalcitol, tacalcitol, falecalcitriol, eldecacitol, inecalcitol, seocalcitol, calcipotriene, maxacalcitol, calcifediol, calcipotriol; synthetic analogs thereof and/or side-chain analogs thereof; and combinations thereof, and an autoantigen derived from MPO, wherein the lipid delivery vehicle comprises POPC, POPG, a PEGylated lipid. For example, the autoantigen derived from MPO may comprise, consist of or consist essentially of an amino acid sequence set forth in any one of SEQ ID NOs 9 and 10.

[0172] The lipid delivery vehicle may comprise a NF- κ B inhibitor selected from the group consisting of calcitriol, calciferol, ergocalciferol, alfacalcidol, doxercalciferol, calcidiol, 22-oxacalcitriol, paricalcitol, tacalcitol, falecalcitriol, eldecacitol, inecalcitol, seocalcitol, calcipotriene, maxacalcitol, calcifediol, calcipotriol; synthetic analogs thereof and/or side-chain

analogs thereof; and combinations thereof, and an autoantigen derived from TSH, wherein the lipid delivery vehicle comprises POPC, POPG, a PEGylated lipid. For example, the autoantigen derived from TSH may comprise, consist of or consist essentially of an amino acid sequence set forth in any one of SEQ ID NOs: 25-55.

[0173] The lipid delivery vehicle may comprise a NF- κ B inhibitor selected from the group consisting of calcitriol, calciferol, ergocalciferol, alfacalcidol, doxercalciferol, calcidiol, 22-oxacalcitriol, paricalcitol, tacalcitol, falecalcitriol, eldecalcitol, inecalcitol, seocalcitol, calcipotriene, maxacalcitol, calcifediol, calcipotriol; synthetic analogs thereof and/or side-chain analogs thereof; and combinations thereof, and an autoantigen derived from Tenascin C, wherein the lipid delivery vehicle comprises POPC, POPG, a PEGylated lipid. For example, the autoantigen derived from Tenascin C may comprise, consist of or consist essentially of an amino acid sequence set forth in any one of SEQ ID NOs: 56-59.

[0174] The lipid delivery vehicle may comprise a NF- κ B inhibitor selected from the group consisting of calcitriol, calciferol, ergocalciferol, alfacalcidol, doxercalciferol, calcidiol, 22-oxacalcitriol, paricalcitol, tacalcitol, falecalcitriol, eldecalcitol, inecalcitol, seocalcitol, calcipotriene, maxacalcitol, calcifediol, calcipotriol; synthetic analogs thereof and/or side-chain analogs thereof; and combinations thereof, and an autoantigen derived from human desmoglein 2, wherein the lipid delivery vehicle comprises POPC, POPG, a PEGylated lipid. For example, the autoantigen derived from human desmoglein 2 may comprise, consist of or consist essentially of an amino acid sequence set forth in SEQ ID NO: 70.

[0175] The lipid delivery vehicle may comprise a NF- κ B inhibitor selected from the group consisting of calcitriol, calciferol, ergocalciferol, alfacalcidol, doxercalciferol, calcidiol, 22-oxacalcitriol, paricalcitol, tacalcitol, falecalcitriol, eldecalcitol, inecalcitol, seocalcitol, calcipotriene, maxacalcitol, calcifediol, calcipotriol; synthetic analogs thereof and/or side-chain analogs thereof; and combinations thereof, and a hybrid insulin peptide (HIP), wherein the lipid delivery vehicle comprises POPC, POPG, a PEGylated lipid. For example, the hybrid insulin peptide (HIP) may comprise, consist of or consist essentially of an amino acid sequence set forth in any one of SEQ ID NOs: 60-65.

[0176] The lipid delivery vehicle may comprise calcitriol and a peptide antigen, wherein the lipid delivery vehicle comprises POPC and POPG. The lipid delivery vehicle may further comprise a PEGylated lipid

[0177] The lipid delivery vehicle may comprise calcitriol and a peptide antigen, wherein the lipid delivery vehicle comprises POPC, POPG and a PEGylated lipid.

[0178] The lipid delivery vehicle may comprise calcitriol and an autoantigen derived from CII, wherein the lipid delivery vehicle comprises POPC and POPG. For example, the autoantigen

derived from CII may be CII₂₅₉₋₂₇₃ antigen (e.g., SEQ ID NO: 1). The lipid delivery vehicle may further comprise a PEGylated lipid.

[0179] The lipid delivery vehicle may comprise calcitriol and an autoantigen derived from PI, wherein the lipid delivery vehicle comprises POPC and POPG. For example, the autoantigen derived from PI may be a PI₃₃₋₆₃ antigen (e.g., SEQ ID NO: 2). The lipid delivery vehicle may further comprise a PEGylated lipid.

[0180] The lipid delivery vehicle may comprise calcitriol and an autoantigen derived from AggreCan, wherein the lipid delivery vehicle comprises POPC and POPG. For example, the autoantigen derived from AggreCan may be an AggreCan₈₄₋₁₀₃Cit93 antigen (e.g., SEQ ID NO: 4). For example, the autoantigen derived from AggreCan may be an AggreCan₈₉₋₁₀₃Cit93 antigen (e.g., SEQ ID NO: 69). The lipid delivery vehicle may further comprise a PEGylated lipid.

[0181] The lipid delivery vehicle may comprise calcitriol and an autoantigen derived from AggreCan, wherein the lipid delivery vehicle comprises POPC and POPG. For example, the autoantigen derived from AggreCan may comprise, consist of or consist essentially of an amino acid sequence set forth in any one of SEQ ID NOs 11-24. The lipid delivery vehicle may further comprise a PEGylated lipid.

[0182] The lipid delivery vehicle may comprise calcitriol and an autoantigen derived from Ro, wherein the lipid delivery vehicle comprises POPC and POPG. For example, the autoantigen derived from Ro may be a Ro₄₀₁₋₄₂₅ antigen (e.g., SEQ ID NO: 5). The lipid delivery vehicle may further comprise a PEGylated lipid.

[0183] The lipid delivery vehicle may comprise calcitriol and an autoantigen derived from Vimentin, wherein the lipid delivery vehicle comprises POPC and POPG. For example, the autoantigen derived from Vimentin may be an Vimentin₄₁₋₈₅ antigen (e.g., SEQ ID NO: 6). In another example, the autoantigen derived from Vimentin may be a Vimentin₄₁₋₈₅Cit64 antigen (e.g., SEQ ID NO: 7). In another example, the autoantigen derived from Vimentin may be a Vimentin₃₈₋₈₈Cit64 antigen (SEQ ID NO: 8). In another example, the autoantigen derived from Vimentin may be a Vimentin₅₃₋₈₅Cit64 antigen (SEQ ID NO: 66). In another example, the autoantigen derived from Vimentin may be a Vimentin₆₀₋₈₅Cit64 antigen (SEQ ID NO: 67). In another example, the autoantigen derived from Vimentin may be a Vimentin₅₉₋₇₁ antigen (SEQ ID NO: 71). The lipid delivery vehicle may further comprise a PEGylated lipid.

[0184] The lipid delivery vehicle may comprise calcitriol and an autoantigen derived from MPO, wherein the lipid delivery vehicle comprises POPC and POPG. For example, the autoantigen derived from MPO may comprise, consist of or consist essentially of an amino acid sequence set forth in any one of SEQ ID NOs 9 and 10. The lipid delivery vehicle may further comprise a PEGylated lipid.

[0185] The lipid delivery vehicle may comprise calcitriol and an autoantigen derived from TSH, wherein the lipid delivery vehicle comprises POPC and POPG. For example, the autoantigen derived from TSH may comprise, consist of or consist essentially of an amino acid sequence set forth in any one of SEQ ID NOs: 25-55. The lipid delivery vehicle may further comprise a PEGylated lipid.

[0186] The lipid delivery vehicle may comprise calcitriol and an autoantigen derived from Tenascin C, wherein the lipid delivery vehicle comprises POPC and POPG. For example, the autoantigen derived from Tenascin C may comprise, consist of or consist essentially of an amino acid sequence set forth in any one of SEQ ID NOs: 56-59. The lipid delivery vehicle may further comprise a PEGylated lipid.

[0187] The lipid delivery vehicle may comprise calcitriol and an autoantigen derived from human desmoglein 2, wherein the lipid delivery vehicle comprises POPC and POPG. For example, the autoantigen derived from human desmoglein 2 may comprise, consist of or consist essentially of an amino acid sequence set forth in SEQ ID NO: 70. The lipid delivery vehicle may further comprise a PEGylated lipid.

[0188] The lipid delivery vehicle may comprise calcitriol and a hybrid insulin peptide (HIP), wherein the lipid delivery vehicle comprises POPC and POPG. For example, the hybrid insulin peptide (HIP) may comprise, consist of or consist essentially of an amino acid sequence set forth in any one of SEQ ID NOs: 60-65. The lipid delivery vehicle may further comprise a PEGylated lipid.

Methods of Preparation

[0189] Suitable methods for the production of a lipid delivery vehicle of the present disclosure will be apparent to the skilled person and/or described herein. For example, a lipid delivery vehicle of the present disclosure may be made using approaches which are well-known in the art of formulation. Suitable lipid delivery vehicles can be formed using mixing processes such as microfluidics, including herringbone micromixing, and T-junction mixing of two fluid streams, one of which contains a peptide antigen, typically in an aqueous solution, and the other of which has the various required lipid components including a NF- κ B inhibitor, typically in ethanol.

[0190] The lipid delivery vehicle may be prepared by combining a phospholipid (such as POPC or POPG, which may be purchased from a variety of commercial sources including Avanti Polar Lipids, Alabaster, AL) and a PEGylated lipid (such as 1,2-dimyristoyl-sn-glycerol methoxypoly ethylene glycol, also known as PEG-DMG 2000K, which may be purchased from a variety of commercial sources including Avanti Polar Lipids, Alabaster, AL) in ethanol. For storage, the lipid delivery vehicles may be suspended within cryoprotectant buffer (e.g., 20 nM tris, 10% sucrose)

and frozen at, for example, $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$. The various lipids may be combined to yield the desired weight ratios and diluted with water and ethanol to a final desired lipid concentration.

[0191] A lipid delivery vehicle comprising a peptide antigen and a NF- κ B inhibitor may prepared by combining the above lipid solution including the NF- κ B inhibitor with a solution comprising the peptide antigen. The lipid solution may be rapidly injected using a NanoAssembler microfluidic system at flow rates between about 3 ml/min and about 18 ml/min into the DNA solution to produce a suspension with a water to ethanol ratio between about 1:1 and about 4:1. The pH of the solution comprising the peptide antigen may be adjusted to at or above the isoelectric point (pI) of the peptide antigen. As will be understood by the skilled person, increasing the pH of a solution to the pI of the peptide antigen will result in a peptide antigen with a neutral charge and increasing the pH of a solution to above the pI of the peptide antigen will result in a peptide antigen with a negative charge.

[0192] A lipid delivery vehicle comprising a peptide antigen and a NF- κ B inhibitor may prepared at room temperature. For example, a lipid delivery vehicle comprising a peptide antigen and a NF- κ B inhibitor may prepared at about 20°C , or about 21°C , or about 22°C , or about 23°C , or about 24°C , or about 25°C , or about 26°C . A lipid delivery vehicle comprising a peptide antigen and a NF- κ B inhibitor may prepared at about 22°C to about 24°C .

[0193] A lipid delivery vehicle comprising a peptide antigen and a NF- κ B inhibitor may prepared below freezing. For example, a lipid delivery vehicle comprising a peptide antigen and a NF- κ B inhibitor may prepared at about -20°C , or about -25°C , or about -30°C , or about -35°C , or between about -20°C to about -30°C . A lipid delivery vehicle comprising a peptide antigen and a NF- κ B inhibitor may prepared at about -28°C to about -32°C .

[0194] As used herein, the term “encapsulation” refers to the process or result of confining one or more payloads or agents, such as a peptide antigen and/or NF- κ B inhibitor, within a lipid delivery vehicle. As used herein, the terms “encapsulation” and “loading” can be used interchangeably. The encapsulation may be within the core of the lipid delivery vehicles and/or within a lipid bilayer and/or between individual lipid bilayers in multilamellar vesicles.

[0195] As used herein, “encapsulation efficiency” refers to the amount of a peptide antigen and/or NF- κ B inhibitor that becomes part of the lipid delivery vehicle, relative to the initial total amount of peptide antigen and/or NF- κ B inhibitor used to prepare the lipid delivery vehicle. For example, if the lipid delivery vehicle contains 92 mg of peptide antigen and/or immunomodulator and 100 mg of peptide antigen and/or immunomodulator was initially provided to form the composition, the encapsulation efficiency may be given as 92%.

[0196] The efficiency of encapsulation of the peptide antigen and/or the NF- κ B inhibitor within the lipid delivery vehicle may be at least 50%, for example about 50%, or 55%, or 60%, or 65%,

or 70%, or 75%, or 80%, or 85%, or 90%, or 91%, or 92%, or 93%, or 94%, or 95%, or 96%, or 97%, or 98%, or 99%. In some examples, the encapsulation efficiency may be at least 80%. In certain examples, the encapsulation efficiency may be at least 90%. In some examples, the encapsulation efficiency may be between 25% to 100%. In some examples, the encapsulation efficiency may be between 50% to 100%. In some examples, the encapsulation efficiency may be between 70% to 100%. In some examples, the encapsulation efficiency may be between 70% to 90%.

Pharmaceutical compositions

[0197] The present disclosure envisages that the lipid delivery vehicle may be utilised in pharmaceutical compositions.

[0198] The pharmaceutical compositions of the present disclosure may comprise one or a plurality of peptide antigens. For example, the pharmaceutical compositions of the present disclosure may comprise one or a plurality of the same peptide antigen. Alternatively, the pharmaceutical compositions of the present disclosure may comprise one or a plurality of different peptide antigens. For example, a pharmaceutical composition of the present disclosure may comprise at least two, or at least three, or at least four, or at least five, or at least six, or at least seven, or at least eight of the same peptide antigen.

[0199] The pharmaceutical compositions of the present disclosure may comprise one or a plurality of lipid delivery vehicles. For example, the pharmaceutical compositions of the present disclosure may comprise one or a plurality of lipid delivery vehicles each comprising different peptide antigens. For example, a pharmaceutical composition of the present disclosure may comprise at least two, or at least three, or at least four, or at least five, or at least six, or at least seven, or at least eight delivery vehicles each comprising different peptide antigens.

[0200] Suitably, in compositions or methods for administration of the lipid delivery vehicle of the disclosure to a subject, the lipid delivery vehicle is combined with a pharmaceutically acceptable carrier as is understood in the art. Accordingly, one example of the present disclosure provides a composition (e.g., a pharmaceutical composition) comprising the lipid delivery vehicle of the disclosure combined with a pharmaceutically acceptable carrier.

[0201] By “pharmaceutically acceptable carrier, diluent or excipient” is meant a solid or liquid filler, diluent or encapsulating substance that may be safely used in systemic administration. Depending upon the particular route of administration, a variety of carriers, diluent and excipients well known in the art may be used. These may be selected from a group including sugars, starches, cellulose and its derivatives, malt, gelatine, talc, calcium sulfate, vegetable oils, synthetic oils, polyols, alginic acid, phosphate buffered solutions, emulsifiers, isotonic saline and salts such as

mineral acid salts including hydrochlorides, bromides and sulfates, organic acids such as acetates, propionates and malonates, water and pyrogen-free water.

[0202] A useful reference describing acceptable carriers, diluents and excipients is Remington's Pharmaceutical Sciences (Mack Publishing Co. N.J. USA, 1991) which is incorporated herein by reference.

[0203] A lipid delivery vehicle of the present disclosure may be administered by any safe route, for example, by oral, rectal, parenteral, sublingual, buccal, intravenous, intra-articular, intramuscular, intra-dermal, subcutaneous, inhalational, intranasal, intraocular, intraperitoneal, intracerebroventricular, topical, mucosal and transdermal administration, although without limitation thereto. For example, the lipid delivery vehicle may be administered parenterally, such as intramuscularly, subcutaneously or intravenously.

[0204] The lipid delivery vehicle may be administered subcutaneously. A pharmaceutical composition administered subcutaneously may be formulated at a pH of between about 7.0 and about 8.0.

[0205] Formulation of a lipid delivery vehicle to be administered will vary according to the route of administration and formulation (e.g., solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suspensions, suppositories, injections, inhalants, gels, microspheres, and aerosols) selected. An appropriate pharmaceutical composition comprising a lipid delivery vehicle to be administered can be prepared in a physiologically acceptable carrier. For solutions or emulsions, suitable carriers include, for example, aqueous or alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles can include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. A variety of appropriate aqueous carriers are known to the skilled artisan, including water, buffered water, buffered saline, polyols (e.g., glycerol, propylene glycol, liquid polyethylene glycol), dextrose solution and glycine. Intravenous vehicles can include various additives, preservatives, or fluid, nutrient or electrolyte replenishers (See, generally, Remington's Pharmaceutical Science, 16th Edition, Mack, Ed. 1980). The compositions can optionally contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents and toxicity adjusting agents, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride and sodium lactate. The lipid delivery vehicle can be stored in the liquid stage or can be lyophilized for storage and reconstituted in a suitable carrier prior to use according to art-known lyophilization and reconstitution techniques.

[0206] The optimum concentration of the active ingredient(s) (i.e., the peptide antigen and immunomodulator) in the chosen medium can be determined empirically, according to procedures known to the skilled artisan, and will depend on the ultimate pharmaceutical formulation desired.

[0207] Upon formulation, compositions of the present disclosure will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically/prophylactically effective. The dosage ranges for the administration of the lipid delivery vehicle of the disclosure are those large enough to produce the desired effect. For example, the pharmaceutical composition may comprise an effective amount of the encapsulated peptide antigen and immunomodulator. Alternatively, or in addition, the pharmaceutical composition may comprise a therapeutically effective amount of the peptide antigen and NF- κ B inhibitor. The pharmaceutical composition may comprise a prophylactically effective amount of the peptide antigen and immunomodulator.

[0208] The dosage should not be so large as to cause adverse side effects. Generally, the dosage will vary with the age, condition, sex and extent of the disease in the patient and can be determined by one of skill in the art. The dosage can be adjusted by the individual physician in the event of any complication.

[0209] Any suitable procedure is contemplated for producing lipid delivery vehicles and/or pharmaceutical compositions described herein.

[0210] Dosage forms include tablets, dispersions, suspensions, injections, solutions, syrups, troches, capsules, nasal sprays, suppositories, aerosols, transdermal patches and the like. These dosage forms may also include injecting or implanting controlled releasing devices designed specifically for this purpose or other forms of implants modified to act additionally in this fashion. Controlled release may be affected by coating with hydrophobic polymers including acrylic resins, waxes, higher aliphatic alcohols, polylactic and polyglycolic acids and certain cellulose derivatives such as hydroxypropylmethyl cellulose. In addition, the controlled release may be affected by using other polymer matrices, liposomes and/or microspheres.

[0211] Compositions may be presented as discrete units such as capsules, sachets, pre-filled syringes, vials, ampoules, functional foods/feeds or tablets each containing a pre-determined amount of one or more therapeutic agents of the disclosure, as a powder or granules or as a solution or a suspension in an aqueous liquid, a non-aqueous liquid, an oil-in-water emulsion or a water-in-oil liquid emulsion. Such compositions may be prepared by any of the methods of pharmacy but all methods include the step of bringing into association one or more agents as described above with the carrier which constitutes one or more necessary ingredients. In general, the compositions are prepared by uniformly and intimately admixing the agents of the disclosure with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product into the desired presentation.

[0212] The above compositions may be administered in a manner compatible with the dosage formulation, and in such an amount as to be effective. The dose administered to a subject, in the context of the present disclosure, should be sufficient to affect a beneficial response in a subject over an appropriate period of time (e.g., generate a tolerogenic immune response). The quantity of agent(s) to be administered may depend on the subject to be treated inclusive of the age, sex, weight and general health condition thereof, factors that will depend on the judgement of the practitioner. In determining the effective dose that may be administered to a subject, inflammation, pro-inflammatory cytokine levels, lymphocyte proliferation, cytolytic T lymphocyte activity and regulatory T lymphocyte function may be evaluated.

[0213] The pharmaceutical compositions of the present disclosure may further comprise a buffer. The buffer may be any suitable buffer known in the art. For example, the buffer may be a TRIS, acetate, glutamate, lactate, maleate, tartrate, phosphate, citrate, carbonate, glycinate, histidine, glycine, succinate and triethanolamine buffer, phosphate buffer. The buffer may be a phosphate buffer. Alternatively, the buffer may be a succinate buffer. The buffer may be a histidine buffer. The buffer may be a citrate buffer.

[0214] The buffer may be selected from US Pharmacopeia (USP) compatible buffers for parenteral use, in particular, when the pharmaceutical formulation is for parenteral use. For example the buffer may be selected from the group consisting of monobasic acids such as acetic, benzoic, gluconic, glyceric and lactic; dibasic acids such as aconitic, adipic, ascorbic, carbonic, glutamic, malic, succinic and tartaric, polybasic acids such as citric and phosphoric; and bases such as ammonia, diethanolamine, glycine, triethanolamine, and TRIS.

[0215] Also described herein is a container comprising a sterile liquid formulation of, or a lyophilised composition comprising, the pharmaceutical composition described herein. Any suitable container known in the art may be used. For example, the container may be selected from the group consisting of a vial, a syringe, an ampoule, a flask, a fermenter, a bioreactor, a bag, a jar, an ampoule, a cartridge and a disposable pen. The container may be a syringe, vial or ampoule.

[0216] The container may be made of glass, metals (e.g., steel, stainless steel, aluminium, etc.) and/or polymers (e.g., thermoplastics, elastomers, thermoplastic-elastomers). The container may be at least partially siliconized. These containers should be sterile.

[0217] A kit or composition may be packaged (e.g. in the same box) with a leaflet including details of the pharmaceutical composition e.g. instructions for administration, details of the antigens and immunomodulators within the composition, etc. The instructions may also contain warnings e.g. to keep a solution of adrenaline readily available in case of anaphylactic reaction following vaccination, etc.

Methods of treatment and prevention

[0218] The lipid delivery vehicles and the pharmaceutical compositions of the present disclosure may be suitable for administration to human or non-human animal subjects, such that the present disclosure provides methods of eliciting a tolerogenic immune response and/or treating and/or preventing an undesirable or deleterious immune response in a subject.

[0219] The present disclosure also provides a composition as described herein for use as a medicament and provides the use of such a composition for the manufacture of a medicament for eliciting a tolerogenic immune response and/or preventing and/or treating an undesirable or deleterious immune response in a subject.

[0220] The present disclosure provides methods of eliciting a tolerogenic immune response and/or treating and/or preventing an undesirable or deleterious immune response in a subject, the method comprising administering a composition as described herein.

[0221] As described herein, an undesirable or deleterious immune response includes, but is not limited to, transplant rejection, allergies, parasitic diseases and an autoimmune disease, disorder or condition and combinations thereof.

[0222] Examples of transplant rejection which can be treated or prevented with the lipid delivery vehicle or the pharmaceutical composition described herein include, but are not limited to, rejections associated with transplantation of stem cells, bone marrow and of organs (e.g., heart, liver, pancreas, kidney, lung, eye, skin etc.) and combinations thereof.

[0223] Examples of allergies which can be treated or prevented with the lipid delivery vehicle or the pharmaceutical composition described herein include, but are not limited to, seasonal respiratory allergies, allergy to aeroallergens (e.g., hay fever), allergy treatable by reducing serum IgE and eosinophilia, asthma, eczema, animal allergies, food allergies, latex allergies, dermatitis, or allergies treatable by allergic desensitisation.

[0224] Examples of an autoimmune disease, disorder or condition which can be treated or prevented with the lipid delivery vehicle or the pharmaceutical composition described herein include, but are not limited to, psoriasis, psoriatic arthritis, ankylosing spondylitis, systemic lupus erythematosus (SLE), myasthenia gravis, stiff-man syndrome, rheumatic heart disease, Sydenham chorea, rheumatoid arthritis, type 1 diabetes, crohn's disease, chronic inflammatory eye diseases including uveitis and birdshot retinopathy, chronic inflammatory lung diseases and chronic inflammatory liver diseases, autoimmune haemolytic anaemia, idiopathic leucopenia, ulcerative colitis, dermatomyositis, scleroderma, mixed connective tissue disease, multiple sclerosis, neuromyelitis optica, vitiligo, alopecia areata, Guillan-Barre syndrome, antiphospholipid syndrome, pernicious anaemia, autoimmune atrophic gastritis, Addison's disease, Goodpasture's syndrome, Behcet's syndrome, Sjogren's syndrome, juvenile inflammatory arthritis, anti-neutrophil-associated cytoplasmic antibody-associated vasculitis, sympathetic ophthalmia,

Hashimoto's disease, hypothyroiditis, celiac disease, dermatitis herpetiformis, demyelinating disease, primary biliary cirrhosis, autoimmune chronic active hepatitis, Graves' disease, hyperthyroiditis, chronic idiopathic thrombocytopenic purpura, pemphigus vulgaris, bullous pemphigoid, anti-neutrophil-associated vasculitis, neuromyelitis optica and checkpoint-inhibitor-induced autoimmune diseases and combinations thereof.

[0225] The present disclosure provides a method of treating and/or preventing an autoimmune disease, disorder or condition in a subject, the method comprising administering a therapeutically effective amount of the lipid delivery vehicle or the pharmaceutical composition described herein, to the subject.

[0226] The present disclosure also provides a method of eliciting a tolerogenic immune response in a subject, said method including the step of administering a therapeutically effective amount of the lipid delivery vehicle or the pharmaceutical composition described herein to the subject.

[0227] The present disclosure also provides the use of the lipid delivery vehicle in the manufacture of a medicament for treating and/or preventing an autoimmune disease, disorder or condition in a subject.

[0228] The present disclosure also provides the lipid delivery vehicle or the pharmaceutical composition described herein for use in treating and/or preventing an autoimmune disease, disorder or condition in a subject.

[0229] As described herein, the term “subject”, “patient” and “individual” includes, but is not limited to, mammals, inclusive of humans, performance animals (e.g., horses, camels, greyhounds), livestock (e.g., cows, sheep, horses) and companion animals (e.g., cats and dogs). For example, the subject may be a human.

[0230] By “elicit a tolerogenic immune response” it is meant to generate or stimulate tolerance of the immune system to a target antigen. The tolerogenic immune response described herein may include exposing antigen-presenting cells (APCs) (e.g., dendritic cells) with a target antigen to induce antigen-specific induction of T cell tolerance. This may lead to T cell clonal deletion, T cell anergy or the proliferation or differentiation of regulatory T cells (Tregs).

[0231] The term “therapeutically effective amount” is the quantity which, when administered, at least partly ameliorates, eliminates or reduces a symptom or pathological sign of an undesirable or deleterious immune response. Alternatively, a therapeutically effective amount is a quantity which, when administered prevents the exacerbation of one or more symptoms or pathological signs of an undesirable or deleterious immune response. The amount to be administered will depend on the characteristics of the subject, such as general health, other diseases, age, sex, genotype, and body weight. A person skilled in the art will be able to determine appropriate dosages depending on

these and other factors. Accordingly, this term is not to be construed to limit the present disclosure to a specific quantity, e.g., weight or amount of compound.

[0232] As generally used herein, the terms “immunise” and “immunisation” refer to methods and/or compositions that are capable of eliciting a tolerogenic immune response against a peptide antigen, whereby subsequent transplant, allergic reaction, parasitic disease or autoimmune disease, disorder or condition is at least partly prevented or minimised.

[0233] As used herein, “treating”, “treat” or “treatment” refers to a therapeutic intervention that at least partly ameliorates, eliminates or reduces a symptom or pathological sign of an undesirable or deleterious immune response, after it has begun to develop. Treatment need not be absolute to be beneficial to the subject. The beneficial effect can be determined using any methods or standards known to the ordinarily skilled artisan. A “prophylactic” treatment is a treatment administered to a subject who does not exhibit signs of an undesirable or deleterious immune response, or exhibits only early signs for the purpose of decreasing the risk of developing a symptom or pathological sign of an undesirable or deleterious immune response. Thus, the methods described herein include prophylactic methods of treatment.

[0234] As used herein, “preventing”, “prevent” or “prevention” refers to a course of action initiated prior to an immune response by exposure to a transplant, allergic reaction, parasitic disease or at risk subject and/or before the onset of a symptom or pathological sign of an undesirable or deleterious immune response, so as to prevent an undesirable or deleterious immune response and/or reduce the symptom or pathological sign. It is to be understood that such preventing need not be absolute to be beneficial to a subject.

[0235] As used herein, an “at risk subject” may refer to a subject that is autoantibody positive and/or has a human leukocyte antigen (HLA) haplotype or a family history that has been identified as susceptible to generating and/or stimulating an undesirable or deleterious immune response.

[0236] For example, a method for treating and/or preventing Sjogren’s syndrome or systemic lupus erythematosus (SLE) in a subject, may comprise administering to the subject a lipid delivery vehicle or a pharmaceutical composition described herein comprising a NF- κ B inhibitor (e.g., selected from the group consisting of calcitriol, calciferol, ergocalciferol, alfacalcidol, doxercalciferol, calcidiol, 22-oxacalcitriol, paricalcitol, tacalcitol, falecalcitriol, eldecacitol, inecalcitol, seocalcitol, calcipotriene, maxacalcitol, calcifediol, calcipotriol; synthetic analogs thereof and/or side-chain analogs thereof; and combinations thereof) and an autoantigen derived from Ro (e.g., Ro401-425 antigen; SEQ ID NO: 5) and to the subject .

[0237] A lipid delivery vehicle described herein comprising a NF- κ B inhibitor (e.g., selected from the group consisting of calcitriol, calciferol, ergocalciferol, alfacalcidol, doxercalciferol, calcidiol, 22-oxacalcitriol, paricalcitol, tacalcitol, falecalcitriol, eldecacitol, inecalcitol, seocalcitol,

calcipotriene, maxacalcitol, calcifediol, calcipotriol; synthetic analogs thereof and/or side-chain analogs thereof; and combinations thereof) and an autoantigen derived from Ro (e.g., Ro₄₀₁₋₄₂₅ antigen; SEQ ID NO: 5), may be used in the manufacture of a medicament for treating and/or preventing Sjogren's syndrome or SLE in a subject.

[0238] A method for treating and/or preventing rheumatoid arthritis in a subject, may comprise administering to the subject a lipid delivery vehicle or a pharmaceutical composition described herein comprising a NF- κ B inhibitor (e.g., selected from the group consisting of calcitriol, calciferol, ergocalciferol, alfacalcidol, doxercalciferol, calcidiol, 22-oxacalcitriol, paricalcitol, tacalcitol, falecalcitriol, eldecacitol, inecalcitol, seocalcitol, calcipotriene, maxacalcitol, calcifediol, calcipotriol; synthetic analogs thereof and/or side-chain analogs thereof; and combinations thereof) and an autoantigen derived from CII (e.g., CII₂₅₉₋₂₇₃ antigen; SEQ ID NO: 1) to the subject.

[0239] A lipid delivery vehicle described herein comprising a NF- κ B inhibitor (e.g., selected from the group consisting of calcitriol, calciferol, ergocalciferol, alfacalcidol, doxercalciferol, calcidiol, 22-oxacalcitriol, paricalcitol, tacalcitol, falecalcitriol, eldecacitol, inecalcitol, seocalcitol, calcipotriene, maxacalcitol, calcifediol, calcipotriol; synthetic analogs thereof and/or side-chain analogs thereof; and combinations thereof) and an autoantigen derived from CII (e.g., CII₂₅₉₋₂₇₃ antigen; SEQ ID NO: 1), may be used in the manufacture of a medicament for treating and/or preventing rheumatoid arthritis in a subject.

[0240] A method for treating and/or preventing rheumatoid arthritis in a subject, may comprise administering to the subject a lipid delivery vehicle or a pharmaceutical composition described herein comprising a NF- κ B inhibitor (e.g., selected from the group consisting of calcitriol, calciferol, ergocalciferol, alfacalcidol, doxercalciferol, calcidiol, 22-oxacalcitriol, paricalcitol, tacalcitol, falecalcitriol, eldecacitol, inecalcitol, seocalcitol, calcipotriene, maxacalcitol, calcifediol, calcipotriol; synthetic analogs thereof and/or side-chain analogs thereof; and combinations thereof) and an autoantigen derived from Vimentin (e.g., Vimentin₄₁₋₈₅; SEQ ID NO: 6, or Vimentin₄₁₋₈₅Cit64 antigen; SEQ ID NO: 7 or SEQ ID NO: 8) to the subject.

[0241] A lipid delivery vehicle described herein comprising a NF- κ B inhibitor (e.g., selected from the group consisting of calcitriol, calciferol, ergocalciferol, alfacalcidol, doxercalciferol, calcidiol, 22-oxacalcitriol, paricalcitol, tacalcitol, falecalcitriol, eldecacitol, inecalcitol, seocalcitol, calcipotriene, maxacalcitol, calcifediol, calcipotriol; synthetic analogs thereof and/or side-chain analogs thereof; and combinations thereof) and an autoantigen derived from Vimentin (e.g., Vimentin₄₁₋₈₅; SEQ ID NO: 6, or Vimentin₄₁₋₈₅Cit64 antigen; SEQ ID NO: 7 or SEQ ID NO: 8), may be used in the manufacture of a medicament for treating and/or preventing rheumatoid arthritis in a subject.

[0242] A method for treating and/or preventing type 1 diabetes in a subject, may comprise administering to the subject a lipid delivery vehicle or a pharmaceutical composition described herein comprising a NF- κ B inhibitor (e.g., selected from the group consisting of calcitriol, calciferol, ergocalciferol, alfacalcidol, doxercalciferol, calcidiol, 22-oxacalcitriol, paricalcitol, tacalcitol, falecalcitriol, eldecacitol, inecalcitol, seocalcitol, calcipotriene, maxacalcitol, calcifediol, calcipotriol; synthetic analogs thereof and/or side-chain analogs thereof; and combinations thereof) and an autoantigen derived from proinsulin (e.g., Proinsulin₃₃₋₆₃ antigen; SEQ ID NO: 2) and to the subject.

[0243] A lipid delivery vehicle described herein comprising a NF- κ B inhibitor (e.g., selected from the group consisting of calcitriol, calciferol, ergocalciferol, alfacalcidol, doxercalciferol, calcidiol, 22-oxacalcitriol, paricalcitol, tacalcitol, falecalcitriol, eldecacitol, inecalcitol, seocalcitol, calcipotriene, maxacalcitol, calcifediol, calcipotriol; synthetic analogs thereof and/or side-chain analogs thereof; and combinations thereof) and an autoantigen derived from proinsulin (e.g., Proinsulin₃₃₋₆₃ antigen; SEQ ID NO: 2), may be used in the manufacture of a medicament for treating and/or preventing type I diabetes in a subject.

[0244] A method for treating and/or preventing an autoimmune disease in a subject, may comprise administering to the subject a lipid delivery vehicle or a pharmaceutical composition described herein comprising a NF- κ B inhibitor (e.g., selected from the group consisting of calcitriol, calciferol, ergocalciferol, alfacalcidol, doxercalciferol, calcidiol, 22-oxacalcitriol, paricalcitol, tacalcitol, falecalcitriol, eldecacitol, inecalcitol, seocalcitol, calcipotriene, maxacalcitol, calcifediol, calcipotriol; synthetic analogs thereof and/or side-chain analogs thereof; and combinations thereof) and an autoantigen derived from aggrecan (e.g., SEQ ID NOs: 4 and 11-24) and to the subject.

[0245] A lipid delivery vehicle described herein comprising a NF- κ B inhibitor (e.g., selected from the group consisting of calcitriol, calciferol, ergocalciferol, alfacalcidol, doxercalciferol, calcidiol, 22-oxacalcitriol, paricalcitol, tacalcitol, falecalcitriol, eldecacitol, inecalcitol, seocalcitol, calcipotriene, maxacalcitol, calcifediol, calcipotriol; synthetic analogs thereof and/or side-chain analogs thereof; and combinations thereof) and an autoantigen derived from aggrecan (e.g., SEQ ID NOs: 4 and 11-24), may be used in the manufacture of a medicament for treating and/or preventing an autoimmune disease in a subject.

[0246] A method for treating and/or preventing Graves' disease in a subject, may comprise administering to the subject a lipid delivery vehicle or a pharmaceutical composition described herein comprising a NF- κ B inhibitor (e.g., selected from the group consisting of calcitriol, calciferol, ergocalciferol, alfacalcidol, doxercalciferol, calcidiol, 22-oxacalcitriol, paricalcitol, tacalcitol, falecalcitriol, eldecacitol, inecalcitol, seocalcitol, calcipotriene, maxacalcitol,

calcifediol, calcipotriol; synthetic analogs thereof and/or side-chain analogs thereof; and combinations thereof) and an autoantigen derived from TSH (e.g., SEQ ID NOs: 25-55) and to the subject.

[0247] A lipid delivery vehicle described herein comprising a NF- κ B inhibitor (e.g., selected from the group consisting of calcitriol, calciferol, ergocalciferol, alfacalcidol, doxercalciferol, calcidiol, 22-oxacalcitriol, paricalcitol, tacalcitol, falecalcitriol, eldecacitol, inecalcitol, seocalcitol, calcipotriene, maxacalcitol, calcifediol, calcipotriol; synthetic analogs thereof and/or side-chain analogs thereof; and combinations thereof) and an autoantigen derived from TSH (e.g., SEQ ID NOs: 25-55), may be used in the manufacture of a medicament for treating and/or preventing Graves' disease in a subject.

[0248] A method for treating and/or preventing anti-neutrophil-associated vasculitis in a subject, may comprise administering to the subject a lipid delivery vehicle or a pharmaceutical composition described herein comprising a NF- κ B inhibitor (e.g., selected from the group consisting of calcitriol, calciferol, ergocalciferol, alfacalcidol, doxercalciferol, calcidiol, 22-oxacalcitriol, paricalcitol, tacalcitol, falecalcitriol, eldecacitol, inecalcitol, seocalcitol, calcipotriene, maxacalcitol, calcifediol, calcipotriol; synthetic analogs thereof and/or side-chain analogs thereof; and combinations thereof) and an autoantigen derived from MPO (e.g., SEQ ID NOs: 9 and 10) and to the subject.

[0249] A lipid delivery vehicle described herein comprising a NF- κ B inhibitor (e.g., selected from the group consisting of calcitriol, calciferol, ergocalciferol, alfacalcidol, doxercalciferol, calcidiol, 22-oxacalcitriol, paricalcitol, tacalcitol, falecalcitriol, eldecacitol, inecalcitol, seocalcitol, calcipotriene, maxacalcitol, calcifediol, calcipotriol; synthetic analogs thereof and/or side-chain analogs thereof; and combinations thereof) and an autoantigen derived from MPO (e.g., SEQ ID NOs: 9 and 10), may be used in the manufacture of a medicament for treating and/or preventing anti-neutrophil-associated vasculitis in a subject.

[0250] A method for eliciting a tolerogenic immune response and/or treating and/or preventing an undesirable or deleterious immune response in a subject, may comprise administering to the subject a lipid delivery vehicle or a pharmaceutical composition described herein comprising a NF- κ B inhibitor (e.g., selected from the group consisting of calcitriol, calciferol, ergocalciferol, alfacalcidol, doxercalciferol, calcidiol, 22-oxacalcitriol, paricalcitol, tacalcitol, falecalcitriol, eldecacitol, inecalcitol, seocalcitol, calcipotriene, maxacalcitol, calcifediol, calcipotriol; synthetic analogs thereof and/or side-chain analogs thereof; and combinations thereof) and an autoantigen derived from Tenascin C (e.g., SEQ ID NOs: 56-59) and to the subject.

[0251] A lipid delivery vehicle described herein comprising a NF- κ B inhibitor (e.g., selected from the group consisting of calcitriol, calciferol, ergocalciferol, alfacalcidol, doxercalciferol, calcidiol,

22-oxacalcitriol, paricalcitol, tacalcitol, falecalcitriol, eldecacitol, inecalcitol, seocalcitol, calcipotriene, maxacalcitol, calcifediol, calcipotriol; synthetic analogs thereof and/or side-chain analogs thereof; and combinations thereof) and an autoantigen derived from Tenascin C (e.g., SEQ ID NOs: 56-59), may be used in the manufacture of a medicament for eliciting a tolerogenic immune response and/or treating and/or preventing an undesirable or deleterious immune response in a subject.

[0252] A method for eliciting a tolerogenic immune response and/or treating and/or preventing an undesirable or deleterious immune response in a subject, may comprise administering to the subject a lipid delivery vehicle or a pharmaceutical composition described herein comprising a NF- κ B inhibitor (e.g., selected from the group consisting of calcitriol, calciferol, ergocalciferol, alfacalcidol, doxercalciferol, calcidiol, 22-oxacalcitriol, paricalcitol, tacalcitol, falecalcitriol, eldecacitol, inecalcitol, seocalcitol, calcipotriene, maxacalcitol, calcifediol, calcipotriol; synthetic analogs thereof and/or side-chain analogs thereof; and combinations thereof) and an autoantigen derived from human desmoglein 2 (e.g., SEQ ID NO: 70) and to the subject.

[0253] A lipid delivery vehicle described herein comprising a NF- κ B inhibitor (e.g., selected from the group consisting of calcitriol, calciferol, ergocalciferol, alfacalcidol, doxercalciferol, calcidiol, 22-oxacalcitriol, paricalcitol, tacalcitol, falecalcitriol, eldecacitol, inecalcitol, seocalcitol, calcipotriene, maxacalcitol, calcifediol, calcipotriol; synthetic analogs thereof and/or side-chain analogs thereof; and combinations thereof) and an autoantigen derived from human desmoglein 2 (e.g., SEQ ID NO: 70), may be used in the manufacture of a medicament for eliciting a tolerogenic immune response and/or treating and/or preventing an undesirable or deleterious immune response in a subject.

[0254] A method for eliciting a tolerogenic immune response and/or treating and/or preventing an undesirable or deleterious immune response in a subject, may comprise administering to the subject a lipid delivery vehicle or a pharmaceutical composition described herein comprising a NF- κ B inhibitor (e.g., selected from the group consisting of calcitriol, calciferol, ergocalciferol, alfacalcidol, doxercalciferol, calcidiol, 22-oxacalcitriol, paricalcitol, tacalcitol, falecalcitriol, eldecacitol, inecalcitol, seocalcitol, calcipotriene, maxacalcitol, calcifediol, calcipotriol; synthetic analogs thereof and/or side-chain analogs thereof; and combinations thereof) and an autoantigen derived from hybrid insulin peptide (HIP) (e.g., SEQ ID NOs: 60-65) and to the subject.

[0255] A lipid delivery vehicle described herein comprising a NF- κ B inhibitor (e.g., selected from the group consisting of calcitriol, calciferol, ergocalciferol, alfacalcidol, doxercalciferol, calcidiol, 22-oxacalcitriol, paricalcitol, tacalcitol, falecalcitriol, eldecacitol, inecalcitol, seocalcitol, calcipotriene, maxacalcitol, calcifediol, calcipotriol; synthetic analogs thereof and/or side-chain

analogs thereof; and combinations thereof) and an autoantigen derived from hybrid insulin peptide (HIP) (e.g., SEQ ID NOs: 60-65), may be used in the manufacture of a medicament for eliciting a tolerogenic immune response and/or treating and/or preventing an undesirable or deleterious immune response in a subject.

[0256] In order that preferred embodiments of the present disclosure may be fully understood and put into practical effect, reference is made to the following non-limiting examples.

[0257] The invention is further disclosed in the following numbered paragraphs:

1. A lipid delivery vehicle comprising a nuclear factor- κ B (NF- κ B) inhibitor and a peptide antigen, wherein the lipid delivery vehicle comprises 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG).
2. The lipid delivery vehicle of paragraph 1, further comprising a PEGylated lipid.
3. The lipid delivery vehicle of paragraph 2, wherein the PEGylated lipid is selected from the group consisting of PEG-modified phosphatidylethanolamines, PEG-modified phosphatidic acids, PEG-modified ceramides, PEG-modified dialkylamines, PEG-modified diacylglycerols, and PEG-modified dialkylglycerols, PEG-c-DOMG, PEG-DMG, PEG-DLPE, PEG-DMPE, PEG-DPPC, PEG-DSPE and combinations thereof.
4. The lipid delivery vehicle of paragraph 2 or 3, wherein the lipid delivery vehicle comprises about 0.5 mol % to about 5 mol % of the PEGylated lipid.
5. The lipid delivery vehicle of any one of paragraphs 1 to 4, wherein the lipid delivery vehicle comprises about 85 mol % to 95 mol % of POPC.
6. The lipid delivery vehicle of any one of paragraphs 1 to 5, wherein the lipid delivery vehicle comprises about 5 mol % to 15 mol % of POPG.
7. The lipid delivery vehicle of any one of paragraphs 1 to 6, wherein the lipid delivery vehicle has a mean diameter of between about 75 nm to about 250 nm, and preferably a mean diameter of between about 80 nm to about 150 nm.
8. The lipid delivery vehicle of any one of paragraphs 1 to 7, wherein the lipid delivery vehicle has a negative surface charge.
9. The lipid delivery vehicle of paragraph 8, wherein the surface charge is less than -15 mV, and preferably less than -20 mV.
10. The lipid delivery vehicle of any one of paragraphs 1 to 9, wherein the lipid delivery vehicle has a polydispersity index of less than 0.20.
11. The lipid delivery vehicle of any one of paragraphs 1 to 10, wherein the lipid delivery vehicle is selected from the group consisting of a liposome, a lipid nanoparticle, a lipid vesicle and a lipid-based particle.
12. The lipid delivery vehicle of paragraph 11, wherein the lipid delivery vehicle is a liposome.

13. The lipid delivery vehicle of any one of paragraphs 1 to 12, wherein the peptide antigen is selected from the group consisting of an autoantigen, an alloantigen and an allergen.

14. The lipid delivery vehicle of paragraph 13, wherein the autoantigen is selected from the group consisting of Ro, collagen type II (CII), proinsulin (PI), insulin, hybrid insulin peptides, chromogranin, aggrecan, islet antigen 2 (IA2), glutamic acid decarboxylase 65-kilodalton isoform (GAD65), glycoprotein (gp70), nuclear antigens, lupus autoantigen, Smith, La, U1-RNP, fibrillin, histones, ribosomal proteins, pyruvate dehydrogenase dihydrolipoamide acetyltransferase (PCD-E2), hair follicle antigens, human tropomyosin isoform 5 (hTM5), human cartilage gp 39 (HCgp39) and gp130-RAPS, dnaJp1, citrullinated proteins, citrullinated peptides, citrullinated type II collagen, citrullinated vimentin, citrullinated fibrinogen, citrullinated aggrecan, citrullinated tenascin C, myelin basic protein, proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG), thyroid stimulating factor receptor (TSH-R), acetylcholine receptor (AchR), gliadin, histones, PLP, glucose-6-phosphate isomerase, thyroglobulin, various tRNA synthetases, proteinase-3, human desmoglein 2 (DSG2) and myeloperoxidase.

15. The lipid delivery vehicle of any one of paragraphs 1 to 14, wherein the peptide antigen comprises, consists of or consists essentially of an amino acid sequence set forth in any one of SEQ ID NOs: 1-59, or a fragment, variant or derivative thereof, or any combination thereof.

16. The lipid delivery vehicle of any one of paragraphs 1 to 15, wherein the peptide antigen comprises, consists of or consists essentially of an amino acid sequence set forth in SEQ ID NO: 2, or a fragment, variant or derivative thereof.

17. The lipid delivery vehicle of any one of paragraphs 1 to 16, wherein the NF- κ B inhibitor decreases the level and/or functional activity of a member of the NF- κ B signalling pathway selected from the group consisting of BTK, LYN, BCR Ig α , BCR Ig β , Syk, Bink, PLC γ 2, PKC β , DAG, CARMA1, BCL10, MALT1, PI3K, PIP3, AKT, p38 MAPK, ERK, COT, IKK α , IKK β , IKK γ , NIK, RelA/p65, P105/p50, cRel, RelB, p52, NIK, Leu13, CD81, CD19, CD21 and its ligands in the complement and coagulation cascade, TRAF6, ubiquitin ligase, Tab2, TAK1, NEMO, NOD2, RIP2, Lek, fyn, Zap70, LAT, GRB2, SOS, CD3 zeta, SIp-76, GADS, ITK, PLC γ 1, PKC θ , ICOS, CD28, SHP2, SAP, SLAM and 2B4.

18. The lipid delivery vehicle of any one of paragraphs 1 to 17, wherein the NF- κ B inhibitor increases the level and/or functional activity of a member of the NF- κ B signalling pathway selected from the group consisting of SHP1, SHIP, PIR-B, CD22, CD72, Fc γ RIIB, I κ B, P100, CTLA4, PD-1, Chi, KIR3DL1, KIR3DL2, KIR2DL and Csk.

19. The lipid delivery vehicle of any one of paragraphs 1 to 18, wherein the NF- κ B inhibitor decreases the level and/or functional activity of a NF- κ B family protein selected from the group consisting of Rel-A (p65), Rel-B, Rel (c-Rel), NF- κ B1 (p50/p105), and NF- κ B2 (p52/p100).

20. The lipid delivery vehicle of any one of paragraphs 1 to 19, wherein the NF- κ B inhibitor is selected from the group consisting of calcitriol, rapamycin, leflunomide, teriflunomide, bevacizumab, bithionol, bortezomib, doxorubicin hydrochloride, cantharidin, carfilzomib, chromomycin daunorubicinum, digitoxin, ectinascidin, emetine, erlotinib hydrochloride, gemcitabine, irinotecan, quinacrine dihydrochloride, fluorosalan, manidipine hydrochloride, narasin, lestaurtinib, ouabain, pemetrexed disodium, sorafenib tosylate, sunitinib malate, tioconazole, topotecan, tribromsalan, triclabendazolum, zafirlukast, withaferin A, quecertin, curcumin, BAY 11-7085 and BAY 11-7082.

21. The lipid delivery vehicle of any one of paragraphs 1 to 20, wherein the NF- κ B inhibitor is calcitriol.

22. The lipid delivery vehicle of any one of paragraphs 1 to 21, wherein the peptide antigen comprises, consists of or consists essentially of an amino acid sequence set forth in SEQ ID NO: 2, or a fragment, variant or derivative thereof, and the NF- κ B inhibitor is calcitriol, or a fragment, variant or derivative thereof.

23. The lipid delivery vehicle of paragraph 22, comprising from about 1 μ g/mL to about 50 μ g/mL of the peptide antigen comprises, consists of or consists essentially of an amino acid sequence set forth in SEQ ID NO: 2, or a fragment, variant or derivative thereof and from about 400 ng/mL to about 600 ng/mL of calcitriol, or a fragment, variant or derivative thereof.

24. The lipid delivery vehicle of any one of paragraphs 1 to 21, wherein the peptide antigen comprises, consists of or consists essentially of an amino acid sequence set forth in any one of SEQ ID NOs: 6-8, or a fragment, variant or derivative thereof, and the NF- κ B inhibitor is calcitriol, or a fragment, variant or derivative thereof.

25. A pharmaceutical composition comprising the lipid delivery vehicle of any one of paragraphs 1 to 24 and a pharmaceutically acceptable carrier.

26. The lipid delivery vehicle of any one of paragraphs 1 to 24 or the pharmaceutical composition of paragraph 25 for use in therapy.

27. A method for eliciting a tolerogenic immune response in a subject, comprising administering to the subject the lipid delivery vehicle of any one of paragraphs 1 to 24 or the pharmaceutical composition of paragraph 25 to the subject.

28. Use of the lipid delivery vehicle of any one of paragraphs 1 to 24 in the manufacture of a medicament for eliciting a tolerogenic immune response in a subject.

29. A method for treating and/or preventing an autoimmune disease, disorder or condition in a subject, comprising administering to the subject the lipid delivery vehicle of any one of paragraphs 1 to 24 or the pharmaceutical composition of paragraph 25 to the subject.

30. Use of the lipid delivery vehicle of any one of paragraphs 1 to 24 in the manufacture of a medicament for treating and/or preventing an autoimmune disease, disorder or condition in a subject.

31. The method of paragraph 29 or the use of paragraph 30, wherein the autoimmune disease is selected from the group consisting of psoriasis, psoriatic arthritis, ankylosing spondylitis, systemic lupus erythematosus (SLE), myasthenia gravis, stiff-man syndrome, rheumatic heart disease, Sydenham chorea, rheumatoid arthritis, type 1 diabetes, Crohn's disease, chronic inflammatory eye diseases including uveitis and birdshot retinopathy, chronic inflammatory lung diseases and chronic inflammatory liver diseases, autoimmune haemolytic anaemia, idiopathic leucopenia, ulcerative colitis, dermatomyositis, scleroderma, mixed connective tissue disease, multiple sclerosis, neuromyelitis optica, vitiligo, alopecia areata, Guillan-Barre syndrome, antiphospholipid syndrome, pernicious anaemia, autoimmune atrophic gastritis, Addison's disease, Goodpasture's syndrome, Behcet's syndrome, Sjogren's syndrome, juvenile inflammatory arthritis, anti-neutrophil-associated cytoplasmic antibody-associated vasculitis, sympathetic ophthalmia, Hashimoto's disease, hypothyroiditis, celiac disease, dermatitis herpetiformis, demyelinating disease, primary biliary cirrhosis, autoimmune chronic active hepatitis, Graves' disease, hyperthyroiditis, chronic idiopathic thrombocytopenic purpura, pemphigus vulgaris, bullous pemphigoid, anti-neutrophil-associated vasculitis, neuromyelitis optica and checkpoint-inhibitor-induced autoimmune diseases.

Examples

Example 1 – Methods

Lipid Delivery Vehicle preparation

[0258] Lipid delivery vehicles (referred to hereinafter in the examples as “liposomes” although this should not be construed as requiring any particular morphology of the lipid delivery vehicles) were prepared using a microfluidic technique with the benchtop NanoAssemblr Ignite instrument (Precision Nano-Systems Inc.). The methanolic lipid phase containing L- α -phosphatidylcholine (EPC): L- α -phosphatidylglycerol (EPG) (35:4 w:w); 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC): 1,2-Dipalmitoyl-sn-glycero-3-phosphoglycerol DPPG (6.3:1.6 w:w); or 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC): 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (POPG) (35:4 w:w); 1,2-Dioleoyloxy-3-trimethylammonium-propane chloride (DOTAP); polyethyleneimine (PEI): POPC:POPG; or DOTAP:POPG-DSPC: Cholesterol were mixed with HEPES buffer inside a microfluidic cartridge at a 1:1 ratio and 12 ml/min flow rate.

[0259] For the drug loaded liposomes, calcitriol (Dishman, Veendaal, the Netherlands) at 2 μ g/mL was added to the lipid phase stream, and peptide [Collagen₂₅₉₋₂₇₃II (GIAGFKGEQGPKGEB; SEQ

ID NO: 1), Proinsulin₃₃₋₆₃ (EAEDLQVGQVELGGGPGAGSLQPLALEGSLQ; SEQ ID NO: 2), Ovalbumin₃₂₃₋₃₃₉ (ISQAVHAAHAEINEAGR; SEQ ID NO: 3), AggreCAN₈₉₋₁₀₃ (ATEGRVRVNSAYQDK; SEQ ID NO: 4), Ro60401-425 (MNVTRTEKDSYVVAFSDEMVPVCPVT; SEQ ID NO: 5), human desmoglein 2 (hDSG2) (SEQ ID NO: 70), Vimentin₄₁₋₈₅ (SEQ ID NO: 6), Vimentin₄₁₋₈₅Cit64 (SEQ ID NO: 7), Vimentin₅₉₋₇₁ (SEQ ID NO: 71)] at 120 µg/mL, 60 µg/mL or 30 µg/mL was added to the HEPES buffer aqueous stream (Table 16). Liposomes were dialysed against HEPES buffer using 10 or 100 kDa cut-off membranes to remove free drug.

[0260] The hydrophilicity of the peptide antigens is assessed. If necessary, to enhance the solubility of the peptide antigen in water, the peptide antigen is diluted with a 2%, 1%, 0.5%, or 0.1% ethanol:HEPES buffer solution. Additionally, the pH of the peptide antigen can be adjusted to make the peptide antigen negatively charged, thereby further increasing its solubility in water.

Surfactant addition to semi-synthetic phospholipid formulations

[0261] 2-Dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (PEG-DMG 2000) varying from 0 to 5 molar % was added to the lipid phase.

Plasma incubation

[0262] Each liposome sample was spiked into pooled healthy human plasma (CryoCheck Pooled Normal Plasma CCN-10, Precision Biologic USA) at a ratio of one volume liposome stock to three volumes plasma. Spiked plasma was incubated at 37°C for 15 minutes, then reconstituted to a total volume of 500 µL in PBS. Size exclusion columns were packed by gravity flow into 10mL nominal volume syringes in-house. These consisted of 10mL Sepharose 4B resin (Merck, Australia) fitted with 30µm top and bottom frits. Columns were equilibrated with at least 1X volume of PBS before use. The sample was then applied to the top of the SEC column and allowed to fully enter the column matrix before PBS was applied stepwise to the top of the column to elute the sample. 22 x 0.5 mL fractions were collected for a total elution volume of 11 mL. Collected fractions were stored at 4°C pending further analyses. Columns were flushed with 2X volumes of PBS + 0.1% Tween-20 to remove any residual sample.

Nanoparticle Tracking Analysis (NTA) to determine peptide antigen/ NF-κB inhibitor retention

[0263] NTA analyses were performed on a NanoSight NS300 instrument, and data processed was using NTA Software v3.4 Build 3.4.4 (Malvern Panalytical, UK). Equal volumes of SEC fractions 6-11 (2.5-5.5 mL elution volume) and fractions 16-22 (7.5-11 mL elution volume) were pooled and thoroughly mixed. Pooled samples were then diluted in PBS to a total volume of 1 mL for NTA measurement. Specific NTA settings were as follows: temperature = 25°C, camera level = 15, syringe pump load = 100, detection threshold = 5, and measurements = 3 x 60 seconds. All other settings were automatically determined by the instrument. Measurements where the average

number of particles detected per frame was ≤ 20 were considered to have negligible particle content above background.

[0264] Calcitriol concentration was measured using a 1,25 Dihydroxy Vitamin D ELISA (Immunodiagnostic Systems) with a sensitivity of 6 pmol/L, according to the manufacturer's instructions which are incorporated herein by reference.

Liposome biodistribution

[0265] BALB/c mice were purchased from OzGene Australian Research Council (BALB/cArc) or Charles River (BALB/cAnNCrl). All mice were kept under specific pathogen-free conditions at the Biological Research Facilities of Translational Research Institute.

^{89}Zr peptide labelling

[0266] Desferrioxamine (DFO)-NH₂ was attached to PI₃₃₋₆₃ COOH using HATU activation, then dialysed to remove free DFO and to avoid metal exposure. DFO attachment was confirmed with MALDI-TOF. The resulting peptide was dissolved after sonication in ethanol. DFO-PI₃₃₋₆₃ was added to ^{89}Zr in HEPES buffer, with a final ethanol concentration of 45%, then incubated at 37°C for 1 hour. After incubating with excess diethylenetriamine pentaacetate (DTPA) to bind free ^{89}Zr , a sample was run on high-performance liquid chromatography (HPLC) to identify ^{89}Zr -DTPA and ^{89}Zr -DFO-NH₂ and Zr-PI₃₃₋₆₃-DFO. The peptide elution time was comparable to cold peptide. ^{89}Zr -DTPA and DFO-NH₂ eluted earlier than cold peptide.

Biodistribution after a single liposome dose in naïve mice.

[0267] Female BALB/c-cArc mice (aged 8 weeks, n=6) and BALB/cAnNCrl mice (aged 18 months, n=7) were used to assess liposome uptake using flow cytometry. Liposomes were labelled with the lipophilic membrane dye DiI (Molecular Probes) at a final concentration of 25 µg/ml for at least 15 minutes at room temperature. Mice received 40 µg QuilA adjuvant at the tail base (subcutaneous; sc) five days prior to liposome injection. Mice then received 100 µl DiI-labelled liposomes (DiI-DEN-181 or DiI-ASITI-201) or PBS in the flank (50 µl each side, sc).

Biodistribution after multiple liposome dosing of proteoglycan-primed mice.

[0268] Male BALB/cAnNCrl mice (n=10) were used to assess liposome uptake using flow cytometry. Liposomes were labelled with the lipophilic membrane dye DiD (Molecular Probes) at a final concentration of 25 µg/ml for at least 15 minutes at room temperature. Mice received 80 µg recombinant human aggrecan G1 proteoglycan (rhG1-PG)-Xa-mFc2a fusion protein emulsified with 2 mg dimethyldioctadecyl-ammonium bromide (DDA; Sigma-Aldrich) intra-peritoneally (ip). Seven, 11 and 14 days later, 100 µl liposomes formulated with POPC/POPG/PEG (empty or containing 20 µg/ml aggrecan₈₉₋₁₀₃ and 400 ng/ml calcitriol) or EPC/EPG (containing 20 µg/ml aggrecan₈₉₋₁₀₃ and 400 ng/ml calcitriol) were injected subcutaneously to the flank. The liposome dose injected on day 14 was labelled with DiD (50 µl each side, subcutaneously).

Flow cytometry

[0269] For flow cytometry analysis, mice were sacrificed with CO₂, and the spleen, liver, lymph nodes (inguinal and axillary), and skin from the injection sites (an area of approximately 1x1 cm) (naïve mice) or lymph nodes (inguinal and axillary) and spleen (rhG1-PG-primed mice) were collected into RPMI containing sodium pyruvate and penicillin-streptomycin-glutamine. The spleen and liver were transferred to 6-well plates containing 1 ml digestion buffer (0.01 mg/ml DNaseI and 1 mg/ml Collagenase D in saline) and injected with an additional 1 ml of digestion buffer. They were then incubated for 30 minutes at 37°C, before being disrupted through a 70 µm strainer and rinsed with saline + 10% fetal bovine serum (FBS). To remove hepatocytes, the liver was first centrifuged at 50xG for 3 minutes, and the immune cell containing-supernatant collected and hepatocyte pellet discarded. The spleen and liver were then both pelleted (1500 rpm for 6 minutes at 4°C), incubated in 1ml ACK lysis buffer for red blood cell lysis (3 minutes at room temperature), and washed in saline ready for staining.

[0270] Left and right skin samples from each mouse were combined into 2 ml saline containing 2mg/ml Dispase II and finely minced with scissors. Samples were then incubated at 37°C on a shaker for 1.5 hours at 200rpm, before being topped up with DNaseI (final concentration 0.01 mg/ml) and Collagenase D (final concentration 1mg/ml) and shaken for a further 45 minutes (37°C). Skin and lymph nodes were then mashed through a 70 µm strainer, washed with saline + 10% FBS and resuspended in saline ready for staining.

[0271] Cells were then transferred to FACS tubes and washed in saline (1500 rpm for 5 minutes at 4°C) before live/dead staining with FVS700 (1/40,000 for 20 minutes at 4°C). Samples were next washed with FACS buffer (1x PBS with 0.4% EDTA and 20mM BSA), incubated with anti-CD16/32 to block Fc receptors (1/200, 10 minutes, 4°C) and then stained with primary antibodies for 20 minutes at 4°C. Cells were then washed with FACS buffer and fixed with 1% PFA (4°C for 40 minutes). Finally, cells were washed twice with FACS buffer, resuspended in 300 µl FACS buffer and acquired on a BD Fortessa the following day.

Fluorescence *in vivo* imaging

[0272] Female BALB/cArc mice (aged 12-14 weeks) were used for *in vivo* imaging experiments (n=27). Liposomes were labelled with lipophilic membrane dye (DiD, Molecular Probes, 25 µg/ml final concentration) as above. Mice were primed with 40 µg QuilA (sc, tail base) 4 days prior to liposome injection. Mice then received 100 µl of either DiD-DEN-181, DiD-ASITI-201, DiD-empty liposome (POPC:POPG:PEG), or PBS on the left flank only (sc). The IVIS Spectrum *in vivo* imaging system (Perkin Elmer) was used to assess *in vivo* distribution of DiD-labelled liposomes in whole body images and subsequently in isolated organs (spleen and liver) and LNs

(inguinal, axillary and mesenteric) at 1, 2, 7 or 14 days post-liposome injection. The total radiant efficiency ($[p/s]/[\mu W/cm^2]$) was calculated for each organ.

Statistical analysis

[0273] All experiments were analysed by one-way (multiple groups) or two-way ANOVA (multiple groups and conditions). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.001$.

Example 2 – Results

Liposome compositions

[0274] Initial experiments were designed using a Design of Experiment (DoE) model (Figure 1) and a microfluidic technique was used to optimise liposomes comprised of either semi-synthetic or synthetic lipids with the following characteristics (target profile), which enhance the passive targeting of phagocytic DC in dLN, improve storage stability without particle agglutination (Moghimi 2006, *Biomaterials*;27(1):136-44; Hoshyar et al. 2016, *Nanomedicine*;11(6):673-92): size 90-160nm, PdI < 0.15 , zeta potential < -50 ; and improve stability (retention) of peptide and calcitriol encapsulation *in vitro* and *in vivo*.

[0275] Several possible semi-synthetic compositions were evaluated. Liposomes encapsulating CII₂₅₉₋₂₇₃ and calcitriol were generated with varying concentrations of DPPC:DPPG:Cholesterol, POPC:POPG or POPC:POPG:MBP-PE, and then tested for 2-week stability of peptide retention *in vitro*.

[0276] For the initial compositions with DPPC:DPPG:Cholesterol, the best CII₂₅₉₋₂₇₃ retention that was achieved was 30% after 2 weeks and 15% after 4 weeks. Peptide retention improved in compositions containing EPC:EPG, POPC:POPG and POPC:POPG:MBP relative to DPPC:DPPG:Cholesterol (Table 1).

Table 1. Comparison of 2 and 4 week stability at -30°C of collagen II₂₅₉₋₂₇₃ in liposomes

No.	Lipid composition (mg/0.5mL liposome volume)	Size (nm)	PdI	Zeta Potential (mV)	CII concentration ($\mu g/mL$ liposome)		
					0w	4w	% rtn at 4w
1	EPC (35):EPG (4)	142.9	0.122	-47.9	80.03	34.78	43.45%
2	DPPC (6.35):DPPG (1.56):CH (2.09)	168.6	0.136	-43.5	83.04	25.01	30.11%
3	POPC (35):POPG (4)	161.4	0.130	-50.5	96.19	46.14	47.98%
4	POPC (35):POPG (4):MBP (1.125)	163.3	0.133	-52.0	46.21	21.89	47.37%

[0277] The stability of PI₃₃₋₆₃ and calcitriol were compared in compositions containing EPC:EPC, POPC:POPG, DPPC:DPPG-Chol, or DOTAP or PEI (Table 2). Size, charge and retention of peptide encapsulation (stability over time) did not meet the target profile in compositions containing PEI, DOTAP, ionisable lipid or DPPC:DPPG-Chol. Size, charge and peptide

encapsulation were adequate for EPC:EPG and POPC:POPG, however peptide retention at 2w was relatively low and calcitriol retention at 2w was low for the EPC:EPG composition.

Table 2. Comparison of size charge and encapsulation at -30°C of PI₃₃₋₆₃ and calcitriol in liposomes of varying composition.

Lipid composition (mg/0.5mL liposome volume)	Size (nm)	PdI	Char ge (mV)	PI ₃₃₋₆₃ concentration (µg/mL)			Calcitriol concentration (ng/mL)		
				0w	2w	% rtn at 4w	0w	2w	% rtn at 4w
EPC (35):EPG (4)	147	0.104	-56.9	29.3	19.4	66	737	514	69.7
DOTAP	29	1.23	1.1	31	ND		ND	ND	
Ionizable lipid	52	0.48	9.2	56	ND		ND		
DPPC(10):DPPG(2):Chol (3)	199	0.189	-53.7	72.6	31.7	44	250	250	100
POPC:POPG:PEI	77	0.2	-33	0.3	ND		ND		
POPG:DSPC:DOTAP-Chol (1.5%)	79	0.26	43.9	34.1	ND		ND		
POPC (35):POPG (4)	235	0.16	-65	10.5	3.8	36	758	704	92.9

ND: not detected. NL: not loaded.

PEG-DMG 2000 surfactant

[0278] The stability of liposomes compositions containing POPC:POPG was compared with between 0 and 5% PEG. Collagen II₂₅₉₋₂₇₃ (SEQ ID NO.1) was poorly retained at 2 weeks in the absence of PEG-DMG 2000. Surprisingly, the highest retention of peptide antigen and calcitriol occurred with a PEG concentration of only 0.5% (Table 3 and 4). Calcitriol retention dropped as PEG concentration increased above 0.5% in PI₃₃₋₆₃ liposomes but not collagen II₂₅₉₋₂₇₃ liposomes.

Table 3. Retention of peptide antigens and calcitriol at -30°C in POPC:POPG liposomes with varying %PEG.

%PEG	Collagen II ₂₅₉₋₂₇₃ (µg/mL)		% retn	PI ₃₃₋₆₃ (µg/mL)		% retn	Calcitriol (ng/mL)		% retn
0	39	3	7.7				391	439	112
0.5	38	10	26.3				852	748	87.8
1	40	11	27.5				777	416	53.5
1.5	43	10	23.2				669	353	52.8
0				35.4	45.4	128	456	475	104
0.5				88.6	92.4	104	530	562	106
2				87.6	91.3	104	456	303	66.4
5				47.7	46.6	97.7	466	549	117

Table 4. Comparison of PI₃₃₋₆₃/calcitriol POPC:POPG liposomes with varying concentrations of PEG, constructed at 60°C or room temperature (RT).

Composition	Tem p (°C)	Size	PdI	Charge	PI ₃₃₋₆₃ (µg/mL)			Calcitriol (ng/mL)		
					0w	2w (4°C)	% retn	0w	2w (4°C)	% retn
POPC-POPG 1.5% PEG	60	117	0.14	-36.2	<LOQ	ND	ND	600	ND	ND

POPC-POPG	RT	235	0.516	-65.5	10.5	3.8	36	758	704	93.0
EPC-EPG	RT	147	0.104	-56.9	29.3	19.4	66.2	737	514	69.7
POPC-POPG 0.5% PEG	RT	122	0.131	-57.0	29.3	22.8	77.8	714	705	98.7
POPC-POPG 0.5% PEG*	RT	115	0.083	-41.6	24.9	23.4	94	438	331	75.6
POPC-POPG 1.5% PEG	RT	115	0.105	-34.5	22.5	10.2	45.3	474	418	88.2

*: post-peptidase assay. LOQ: limit of quantification.

Table 5. Retention of Ro60₄₀₁₋₄₂₅ and calcitriol at -30°C POPC:POPG liposomes with varying %PEG

%PEG	Size (nm)	PdI	Zeta (mV)	SJ45 (µg/mL)		%retn	D3 (ng/mL)		%retn
0%PEG	93	0.139	-54	22	19	86	823	788	96
0.5%PEG	90	0.170	-40	49	63	130	817	601	74
1.5%PEG	79	0.197	-40	42	31	75	558	359	64

Table 6. Retention of Vimentin₃₈₋₈₈Cit64 and calcitriol at -30°C POPC:POPG liposomes with varying %PEG

%PEG	Size (nm)	PdI	Zeta (mV)	Vimentin ₄₁₋₈₅ (µg/mL)		%retn	D3 (ng/mL)		%retn
0.5%PEG	91	0.145	-27	22	21	95	1041	652	63
1.5%PEG	97	0.189	-45	23	23	100	1212	1145	94

Table 7. Retention of Vimentin₄₁₋₈₅Cit64 and calcitriol at -30°C POPC:POPG 0.5% PEG liposomes

Stability week	Size (nm)	PdI	Zeta (mV)	Vimentin ₄₁₋₈₅ Cit64 (µg/mL)		%retn	D3 (ng/mL)		%retn
Week 0	94	0.150	-19	29	30	75	910	883	88
Week 1	92	0.186	-25	35	29	75	888		88
Week 3	102	0.224	-13.2	29	24	66	962		96

Table 8. Retention of PI₃₃₋₆₃ and calcitriol at -30°C in POPC:POPG liposomes with 0.5 %PEG.

Stability week	Size (nm)	PdI	Zeta (mV)	PI33-63 (µg/mL)	%retn	D3 (ng/mL)	%retn
Week 0	122	0.131	-57.0	29.3	43	714	89
Week 6	113	0.139	-54.0	22.2	37	567	71

Table 9. Retention of PI₃₃₋₆₃ and calcitriol at -30°C EPC:EPG liposomes

Stability week	Size (nm)	PdI	Zeta (mV)	PI33-63 (µg/mL)	%retn	D3 (ng/mL)	%retn
Week 0	147	0.104	-56.9	29.3	49	737	70
Week 6	144	0.128	-59	19.4	32	643	70

Table 10. Retention of PI₃₃₋₆₃ and teriflunomide at -30°C POPC:POPG 0.5% PEG liposomes

Stability week	Size (nm)	PdI	Zeta (mV)	PI33-63 (µg/mL)	%retn	Teriflunomide (ng/mL)	%retn
Week 0	123	0.124	-30.8	25	100	561	63
Week 2	116.5	0.149	N/A	18	80	447	50
Week 4	109.3	0.12	-36	16	64	395	45

Table 11. Retention of Vimentin₄₁₋₈₅Cit64 and calcitriol at -30°C POPC:POPG 3% PEG liposomes

Stability day	Size (nm)	PdI	Zeta (mV)	Vimentin ₄₁₋₈₅ Cit64 (µg/mL)		%retn	D3 (ng/mL)		%retn
Day 1	79	0.123	-11	22	23	90	1.22	0.88	100
Day 3	77	0.109	-17	25	25	100	676	681	68

Differentiation of free or surface-adhered peptide and liposome-encapsulated peptide

[0279] An important aspect to consider when building a nanocarrier is the level of protection that the nanoparticle provides the encapsulated API (e.g., peptide antigen and NF- κ B inhibitor), as this will influence the integrity of the payload at the targeted tissue. Ideally, antigenic peptides should be protected by encapsulation within the liposomes to avoid possible triggering of immune-complex-mediated immune responses. Unprotected peptide may be degraded by exposure to tissue or blood proteases so that it is no longer an effective tolerogen. Furthermore, residual unencapsulated peptide or surface-adsorbed peptide may lead to false assumptions about the concentration of liposome-encapsulated peptide. Prior to scale-up, free peptide is generally removed after liposome production by dialysis against HEPES buffer. At scale, peptide is removed by trans-flow filtration, which subjects liposomes to greater sheer stress, and surface-adsorbed peptide may not remain with the liposomes.

[0280] The inventors compared peptide retention of PI₃₃₋₆₃/calcitriol POPC:POPG 1.4% PEG liposomes pre and post dialysis through a 10 kDa or a 100 kDa membrane. As PI₃₃₋₆₃ is approximately 3 kDa, the larger pore-size would be required to remove peptide aggregates. The data demonstrate that 17% of peptide was removed by the 10 kDa membrane compared with overnight storage in PBS. In contrast >80% of peptide was removed by the 100 kDa membrane, suggesting that aggregated peptide had adsorbed to the liposome surface. Reducing the temperature of lipid incorporation into the methanol phase and mixing with peptide at room temperature rather than 60°C enhanced peptide retention in liposomes, even with 100 kDa dialysis (Table 4). The inventors found that while the optimal PEG concentration varies, the presence of PEG *per se* improves the stability of the lipid delivery vehicle (Tables 3 to 11).

[0281] To validate the concentration of liposome-encapsulated peptides, the inventors developed an *in vitro* protease digestion assay that is based on the concept that unincorporated or surface-adsorbed peptides would be digested by specific protease *in vitro*, while peptides in the aqueous core would be protected from protease digestion. After protease incubation, liposomal peptide was quantified by liquid chromatography-tandem mass spectrometry (LC-MS/MS). PI₃₃₋₆₃ was digested with chymotrypsin.

[0282] Consistent with the *in vitro* stability data for PI₃₃₋₆₃/calcitriol liposomes (Table 3), a greater percentage of peptide (74%) was encapsulated within POPC:POPG 0.5% PEG-DMG 2000 liposomes than with POPC:POPG alone (44%) or POPC:POPG 1.4% PEG (64%) (Table 12). The positive control, PI₃₃₋₆₃ naked peptide was completely degraded by the protease chymotrypsin during the digestion.

Table 12. Chymotrypsin digestion determines encapsulated liposomal PI₃₃₋₆₃

Liposome composition	PI ₃₃₋₆₃ (µg/mL)			
	before digestion	after digestion	reduction	
POPC:POPG 1.4% PEG	20.66841	13.23077	7.43764	36
POPC:POPG 0.5% PEG	17.18908	12.73158	4.45750	26
POPC:POPG	25.7204	11.3051	14.4153	56
Naked PI ₃₃₋₆₃	32.25898	0.02451	32.23447	100

Estimating *in vivo* stability in plasma

[0283] To be able to assess the effect of plasma on stability, the inventors developed an assay to assess liposome retention of peptide and calcitriol after brief incubation with plasma or PBS. After 100 kDa dialysis against HEPES buffer, particles were incubated with plasma for 15 min then fractionated according to size using size exclusion chromatography (SEC), based on methods developed for purification of extracellular vesicles (Lane et al. Methods Mol Biol. 2017;1660:111-30). Particles in the expected size range for liposomes (~100 nm as measured using NanoSight) were recovered in Fractions 6 to 11, while free peptide but almost no liposomes were collected in Fractions 16-22 (Figure 2). After collecting fractions, peptide and calcitriol concentrations were quantified using mass spectrometry and a sensitive ELISA respectively, to calculate retention per fraction (Fractions 6-11, Fractions 16-22) relative to the total peptide and calcitriol recovered from all fractions (Fractions 6-22).

[0284] To test the effect of plasma incubation, PI₃₃₋₆₃/calcitriol/0.5%PEG liposomes were incubated with plasma or PBS for 15 min or 60 min then fractionated by SEC and quantified peptide and assessed particles by NTA. Fewer particles were recovered, and a lower % peptide retention rate occurred as the duration of incubation in plasma increased (Table 13, Figure 3).

Table 13. Retained peptide in POPC:POPG:PI₃₃₋₆₃/calcitriol liposomes after SEC assay

Sample	Peptide recovered F6-11 (ng)	Peptide Recovered F1-22 (ng)	Retention F6-11 (%)
Liposomes in PBS, 15 minutes	229.0	351.2	65.2
Liposomes in Plasma, 15 minutes	191.6	425.8	45
Liposomes in Plasma, 60 minutes	69.7	351.8	19.8

[0285] To determine retention of peptide and calcitriol after SEC, PI₃₃₋₆₃/calcitriol/0.5%PEG liposomes, produced during scale-up with a 12 mL/min TFF flow rate, was incubated with plasma 15 min then collected all fractions by SEC and calculated % encapsulated peptide and calcitriol in F6-11 relative to total recovered. 50.5% of peptide and 33% calcitriol was retained in F6-11 after plasma incubation and separation by SEC (Table 14).

[0286] In a protease assay carried out on the same liposomes, peptide retention was 48% and calcitriol retention 100% (Table 14). Together these data indicate that peptide retention estimated using SEC and peptidase assay is very similar. Thus, it is likely that both assays separate liposome-encapsulated from free or liposome adherent peptide. Calcitriol retention is not affected by

protease incubation but is progressively reduced by plasma incubation. This is expected due to the presence of vitamin D binding protein (DBP) in plasma (Chun et al. Front Endocrinol (Lausanne). 2019;10:718), which binds vitamin D with high avidity. DBP will bind and remove calcitriol exposed within the liposome bilayer. Calcitriol bound to DBP is inactive in most cells. Although the concentration of DBP is likely to be lower in subcutaneous tissue than plasma, some loss of liposomal calcitriol activity en route to dLN would be expected after subcutaneous administration and should be accounted for in biodistribution and toxicokinetic studies (Sonigra et al. 2022, JCI Insight 2022;7:e160964). It should be noted that commercial calcitriol assays measure total calcitriol including DBP-bound and free calcitriol. DBP-calcitriol is taken up and metabolised by epithelial cells in the proximal tubule (Chun et al. Front Endocrinol (Lausanne). 2019;10:718).

Table 14. Retained peptide and calcitriol in POPC:POPG:PI₃₃₋₆₃/calcitriol liposomes: SEC estimate of *in vivo* stability in plasma

Sample	Peptide in F6-11 (ng)	Peptide in F1-22 (ng)	Retention in F6-11 (%)
POPC: POPG 0.5% PEG	492.9	970.9	50.8
	Calcitriol in F6-11 (pmol)	Calcitriol in F12-22 (pmol)	% Calcitriol Retention F6-11
POPC: POPG 0.5% PEG	1.00	3.04	32.8

Table 15. Retained peptide and calcitriol in POPC:POPG:PI₃₃₋₆₃/calcitriol liposomes: post-peptidase incubation

Sample	Size	PdI	Peptide	Encapsulated peptide (%)	Encapsulated Calcitriol (%)
POPC: POPG 0.5% PEG	104	0.06	21.6	10.4 (48)	593 (100)

Table 16. POPC:POPG liposomes with various peptide antigens

SEQ ID NO	Isoelectric point (pH)	Net Charge at pH7	MW (g/mol)	PEG (% mol/mol)	Size (nm)	PdI	Liposome charge
1	6.98	0	1487.61	0.5	119	0.17	-43
2	0.57	-5	3020.26	0.5	150	0.10	-50
6	11.81	+5	4363.83	0.5	88	0.16	-39
7	11.54	+4	4364.83	3	80	0.10	-17
69	9.79	+1	1693.82	0.5	114	0.16	-24
70	6.99	0	1918.07	0.5	145	0.16	-15
71	11.82	+3	1435.63	0.5	94	0.17	-40

Biodistribution

[0287] To assess performance *in vivo*, the biodistribution of EPC/EPG/peptide/calcitriol (DEN-181, e.g., as described in Sonigra et al. JCI Insight 2022;7:e160964) liposomes with POPC/POPG/0.5%PEG/peptide/calcitriol (ASITI-201) and POPC/POPG/0.5%PEG (empty) liposomes was compared. Liposomes were fluorescent-labelled with lipophilic dye DiD and administered subcutaneous to the tailbase of naïve mice, 4 days after local injection with QuilA adjuvant to enlarge the dLN for flow cytometry. *In vivo* imaging (using IVIS Spectrum) was used to quantify radiant intensity in the whole body and individual lymphoid organs. PBS was injected

as a control. Whole body imaging identified signal at the skin injection site of all mice administered liposomes but not PBS, at 1 day and 2 day post-injection. No signal was observed at day 7 and day 14. Signal was similarly observed in individual organs until day 2 but not day 7 or 14. Figure 4 shows the results of radiant intensity quantification in 2-3 mice per group that received liposomes, with PBS background subtracted. No radiant intensity was observed in the skin or dLN of the non-injected side (not shown). Skin site DiD intensity was maximal at day 1, decreasing at day 2 in all groups.

[0288] Surprisingly, DiD intensity of ASITI-201 (POPC/POPG/0.5%PEG/peptide/calcitriol) was higher at day 1 in both dLN than either DEN-181 (EPC/EPG/peptide/calcitriol) or empty liposomes (POPC/POPG/0.5%PEG) (Figure 4). This suggests that both the ASITI-201 liposome formulation and its payload enhanced uptake in dLN. Uptake of ASITI-201 was also lower than that of DEN-181 in the liver suggesting a beneficial effect of the liposome composition on dLN targeting relative to systemic lymphoid organs. Also surprisingly, DiD intensity of empty liposomes continued to increase in dLN and liver by day 2, while DiD intensity of DEN-181 and ASITI-201 decreased (Figure 4). Given that topical skin application of vitamin D was shown to reduce the number of DC in skin and enhance DC migration to dLN (Gorman et al. J Steroid Biochem Mol Biol. 2010;121(1-2):247-9), the inventors hypothesize that subcutaneous administration of liposomes containing calcitriol synergistically increases the infiltration and uptake of liposomes by migratory DCs in dLN compared with a relatively delayed empty liposome uptake by steady state migratory dLN DCs.

[0289] To assess peptide biodistribution, PI₃₃₋₆₃ was conjugated with DFO-NH₂ then labelled with ⁸⁹Zr as described herein. It was then formulated into POPC/POPG/0.5%PEG/PI₃₃₋₃₆/calcitriol liposomes and dialysed. After subcutaneous administration of liposomes or free radiolabelled peptide to the left flank of BALB/c mice, images were taken 2 weeks later using PET-CT on live mice (Figure 5C). Radio-HPLC showed 87.6% of ⁸⁹Zr was bound to DFO-PI₃₃₋₃₆. Liposome characteristics were comparable to liposomes generated with cold PI₃₃₋₃₆. After liposome injection, a high intensity of radiation at the skin injection site was observed, the left axillary and inguinal dLNs. Signal was first detected at 2 hours and was retained at these sites for 14 days (the maximum detectable half-life for ⁸⁹Zr). Given that fluorescent lipid signal disappeared after 2 days, the retention of peptide in skin and dLNs after liposomal delivery was highly surprising (Figure 5C). Liposomal peptide retention in dLNs was much more effective than peptide alone, as radiolabelled peptide delivered subcutaneous was observed in the skin but not dLNs (Figure 5B). For mice injected with liposomes or naked peptide, radiation signal was also detected in the kidney, the expected clearance site for radiolabelled chemicals.

Cellular uptake

[0290] To determine the cells taking up liposomes, either ASITI-201 or DEN-181 were labelled with fluorescent dye DiI, administered subcutaneously to naive mice, then the proportions of DiI⁺ DCs, monocytes and B cells were quantified by flow cytometry in at the skin injection site, dLN, liver and spleen. No DiI staining was observed above background in spleen. 20% of all live cells in skin took up liposomes, including 70% of CD8⁺ DCs, 40-60% of CD301b⁺ DCs and 90% of CD301b⁺ dermal DCs.

[0291] Antigen-presenting CD301b⁺ dDCs were shown to induce antigen-specific Tregs through retinoic-acid induced ALDH, and to be required for the control of Tfh during immunisation (Weckel et al., Immunity. 2023;56(6):1239-54 e7; Kumamoto et al. Elife. 2016;5). In dLN, uptake of liposomes varied between mice but averaged 5% pDCs, 10% CD8⁺ cDC1, 5% CD301b⁺ cDC2 and 2% CD301b⁺ cDC2. In the liver 9-14% of CD301b⁺ DCs were DiI⁺, while small proportions of pDCs, CD8⁺ DCs and CD301b⁺ DCs were DiI⁺. These data show that ASITI-201 and DEN-181 liposomes are taken up by skin, dLN and liver DCs with a bias towards tolerogenic CD301⁺ dermal DCs in skin and liver (Figure 6).

Conclusion

[0292] POPC/POPG/0.5%PEG/peptide/calcitriol demonstrated significantly better peptide encapsulation and superior stability *in vitro*, using multiple assays, than any other liposome composition. These liposomes are highly suited to scale up and manufacture. *In vivo*, POPC/POPG/0.5%PEG/peptide/calcitriol liposomes demonstrated better and more rapid targeting of dLN than EPC/EPG/peptide/calcitriol liposomes or empty POPC/POPG/0.5%PEG liposomes, suggesting that this lipid composition synergises with calcitriol to enhance that targeting of dLN, thus minimising off-target effects. After subcutaneous administration of POPC/POPG/0.5%PEG/peptide/calcitriol liposomes, peptide was retained for presentation by DCs in skin and dLNs for at least 14 days (Figure 5B and 5C). The desired target DCs, including CD301b⁺ DCs take up liposomes in skin, dLN and liver (Figure 6).

Example 3 – In vivo peptide biodistribution

[0293] To compare peptide biodistribution after administration of liposomes comprising different compositions, PI₃₃₋₆₃ (SEQ ID NO. 2) was conjugated with DFO-NH₂ then labelled with ⁸⁹Zr as described herein.

Methods

Synthesis of PI₃₃-DFO

[0294] A solution of PI₃₃₋₆₃ (1.0 mg, 0.33 μmol) in 0.1M NaHCO₃ (500 μL) was prepared in a 1.5 mL Eppendorf tube. A solution of p-SCN-deferoxamine (1.24 mg, 1.65 mmol) in dimethyl sulfoxide (DMSO) (300 μL) was gently heated and then transferred to a reaction vessel. The tube was placed into an Eppendorf ThermoMixer® and agitated at 37°C for 16 hours. The crude mixture

was purified by semi-preparative HPLC using standard methods. The fractions were collected and lyophilised resulting in PI₃₃-DFO, a white solid.

Radiolabelling of peptide

[0295] 1M HEPES (pH 7.4, 188 µL) was added to PI₃₃-DFO (192.4 µL, 96 µg) followed by buffered ⁸⁹Zr (120 µL, 42 MBq). The reaction mixture was briefly centrifuged to ensure uniformity of the mixture and then incubated at 37°C and 500 rpm for 60 minutes. An aliquot of the solution (5 µL) was added to 5 mM EDTA (5 µL) and analysed by RP-HPLC 5-100% MeCN (0.1% TFA) over 15 minutes. The conjugated and labelled PI₃₃₋₃₆ was then formulated into POPC/POPG/0.5%PEG/PI₃₃₋₆₃/calcitriol or EPC/EPG/PI₃₃₋₆₃/calcitriol liposomes and dialysed.

[0296] Mice were subcutaneously injected with approximately 150 kBq of radioactivity at the base of the tail. This procedure ensured that the amount of injected activity was consistent across different compounds, accounting for variations in their specific activity. After injection at the base of tail, liposome uptake was expected in draining left and right inguinal lymph nodes. Proportionately more lymph node uptake may occur on one side depending on the site of the subcutaneous liposome depot.

[0297] Longitudinal PET-CT scans were performed at days 3, 5, 7, and 11 post-injection on live mice, with *ex-vivo* biodistribution analysis conducted on days 3 and 11.

Results

[0298] Radio-HPLC showed 97-99% of ⁸⁹Zr was bound to DFO-proinsulin peptide. Liposome characteristics were comparable to liposomes generated with cold PI₃₃₋₃₆. After administration of POPC/POPG/0.5%PEG/PI₃₃₋₆₃/calcitriol liposomes, radiation in left and right inguinal and surprisingly also the left and right axillary dLN was significantly higher at 3, 5 and 11 days than for peptide alone (Figure 7A-D).

[0299] Radiation was low at all time points in the axillary dLN after administration of EPC/EPG/PI₃₃₋₆₃/calcitriol liposomes and was not significantly higher than peptide alone in axillary dLNs at any time point (Figure 7C and 7D). After administration of EPC/EPG/PI₃₃₋₆₃/calcitriol liposomes, radiation was higher than peptide alone only in the right inguinal dLN (Figure 7B). After POPC/POPG/0.5%PEG/PI₃₃₋₆₃/calcitriol or EPC/EPG/PI₃₃₋₆₃/calcitriol liposome administration, radiation at the skin injection site was higher at 4 hours and 3 days than after administration of peptide alone. By 3 days, radiation was significantly lower at the injection site after POPC/POPG/0.5%PEG/PI₃₃₋₆₃/calcitriol than EPC/EPG/PI₃₃₋₆₃/calcitriol liposomes, reflecting a greater proportion present in the dLNs. Biodistribution data at day 3 confirmed greater distribution of peptide in the axillary dLNs after POPC than EPC liposomes. Biodistribution was even more striking at day 11, wherein the peptide was distributed at higher levels to all dLNs after POPC/POPG/0.5%PEG/PI₃₃₋₆₃/calcitriol than EPC/EPG/PI₃₃₋₆₃/calcitriol liposomes. After

POPC/POPG/0.5%PEG/PI₃₃₋₆₃/calcitriol liposomes, peptide was distributed at very low levels in liver, spleen and kidney at day 3. Peptide distribution to the kidney for excretion was significantly lower after POPC/POPG/0.5%PEG/PI₃₃₋₆₃/calcitriol than for EPC/EPG/PI₃₃₋₆₃/calcitriol liposomes or peptide at day 3.

Conclusion

[0300] Peptide persists significantly longer in skin and dLNs when encapsulated in liposomes than when delivered free. Surprisingly, POPC/POPG/PEG liposomal peptide migrates significantly further to distal (axillary) dLNs within 3 days and persists at significantly higher levels for longer in the dLNs and less in the kidney than peptide within the EPC/EPG liposomes (Figure 7). These data are consistent with POPC/POPG/PEG/peptide/calcitriol liposomes' better stability and retention of peptide *in vitro* as compared to EPC/EPG/peptide/calcitriol liposomes.

Example 4 – Liposome uptake by antigen-presenting cells in vivo

[0301] To compare liposome uptake by and impact on antigen presenting cells (APCs) after multiple subcutaneous administrations of liposomes of different compositions in the early stages of the proteoglycan induced mouse model of arthritis (PGIA) (e.g., as described in Galea R et al. *JCI Insight* 2019, 4(18):e126025) liposomes were prepared with POPC/POPG/0.5%PEG/aggrecan₈₉₋₁₀₃/calcitriol, EPC/EPG/aggrecan₈₉₋₁₀₃/calcitriol and POPC/POPG/0.5%PEG (empty) and compared. After priming with recombinant human phosphatidylglycerol (rh-PG), liposomes were administered subcutaneously to the flank on days 7, 11 and 14. On day 14, the liposomes were fluorescently labelled with lipophilic dye DiD, Mice were sacrificed on day 15 and axillary and inguinal dLN and spleen were analysed by flow cytometry (Figure 8A). Aggrecan₈₉₋₁₀₃ corresponds to SEQ ID NO. 69.

Results

[0302] Surprisingly, in the dLN, uptake of POPC/POPG/aggrecan/calcitriol liposomes and often also POPC/POPG empty liposomes was significantly higher than uptake of EPC/EPG/aggrecan/calcitriol liposomes in total CD19⁺, memory and germinal centre B cells as well as all DC subsets and other myeloid cells (Figure 8B). In the spleen, uptake of POPC/POPG/aggrecan/calcitriol liposomes and POPC/POPG empty liposomes was significantly higher than uptake of EPC/EPG/aggrecan/calcitriol liposomes but only in cDC1, cDC2 dendritic cells and other myeloid cells (Figure 8C).

Conclusions

[0303] After multiple subcutaneous doses of liposomes at the early stages of an animal model of arthritis, liposome uptake by antigen presenting B cells and DCs in dLN is higher when the lipid composition contains POPC/POPG/PEG than EPC/EPG. In the spleen this is only the case for DCs. Consistent with the radiolabelled peptide biodistribution studies, these uptake data in dLNs

confirm that targeting of dLNs is more effective with POPC/POPG/PEG than EPC/EPG liposome compositions. Identification of DiD-label in splenic DCs in the absence of peptide biodistribution to the spleen suggests that after liposome uptake, dLN DCs process their peptide payload for antigen presentation and traffic from dLN to spleen, facilitating a more widespread mechanism of action after POPC/POPG/PEG than EPC/EPG liposomes.

Example 5 – Flow cytometric analysis of antigen presenting cell function and T cell response

Methods

[0304] Draining LN samples from Example 4 were stained with a panel of markers to identify CD19⁺ B cells, CD11c⁺ conventional DCs and CD11c⁻ plasmacytoid (p)DCs as well as the levels of MHC class II, CD80, CD86 and PD-L1 on cells expressing DiD (e.g. as published in Galea R et al. JCI Insight 2019, 4(18):e126025), then analysed by flow cytometry. Splenocytes were restimulated with AggreCan₈₉₋₁₀₃ peptide. The following day, antigen-activated CD4⁺ T cells expressing CD154 were identified and their expression of IFN- γ and TNF compared in each group of mice (Figure 9 panel A).

Results

[0305] In dLN, myeloid and plasmacytoid DiD⁺ DCs expressed lower levels of MHC class II (Figure 9A and 9B) and memory and germinal centre B cells expressed higher levels of MHC class II (Figure 9C and 9D) after uptake of POPC/POPG/calcitriol/aggreCan than EPC/EPG/calcitriol/aggreCan liposomes. PD-L1 expression also differed between myeloid and plasmacytoid DCs after uptake of POPC/POPG/calcitriol/aggreCan liposomes compared to EPC/EPG/calcitriol/aggreCan liposomes (Figure 9A and 9B).

[0306] Among splenic T cells, the frequency of cells responding to aggreCan peptide restimulation in vitro by CD154 expression was equivalent in mice administered POPC/POPG/calcitriol/aggreCan or EPC/EPG/calcitriol/aggreCan liposomes (Figure 9E), but IFN- γ or IFN- γ and TNF production by CD154⁺ antigen-reactive T cells was significantly lower in mice administered POPC/POPG/calcitriol/aggreCan liposomes compared to EPC/EPG/calcitriol/aggreCan liposomes (Figure 9F and 9G).

Conclusion

[0307] In antigen-primed mice in the early stages of inflammatory arthritis, multiple doses of POPC/POPG/calcitriol/aggreCan liposomes are significantly more suppressive of dendritic cell MHC class II, and antigen-reactive T cells secrete lower levels of pro-inflammatory cytokines than in mice administered multiple doses of EPC/EPG/calcitriol/aggreCan liposomes. This is consistent with the better uptake of POPC/POPG/calcitriol/peptide liposomes and their payload due to greater stability of the liposomes.

Example 6 – Ex vivo calcitriol biodistribution

Methods

[0308] Tritiated calcitriol ([³H]Calcitriol) was purchased from American Radiolabeled Chemicals (St Louis, MO) at 20 Ci/mmol specific activity. 8 μ Ci of this was spiked into an excess (>10-fold by mass) of unlabelled calcitriol and incorporated into POPC/POPG/0.5%PEG/PI₃₃₋₆₃/calcitriol or EPC/EPG/PI₃₃₋₆₃/calcitriol liposomes. Liposomal formulations were then dialysed overnight against HEPES buffer, recovered and the specific activity of the resulting formulation determined via scintillation counting of a 10 μ L aliquot.

[0309] Male BALB/c mice (8 weeks of age, n=3 per timepoint per compound) were each administered 100 μ L of the [³H]Calcitriol labelled liposomal formulations (Approximately 0.25 μ Ci) as a subcutaneous injection near the base of the tail. Mice were then sacrificed at 1 hour and 72 hours post administration to assess biodistribution. 50 μ L blood was collected by cardiac puncture, inguinal and axillary lymph nodes as well as all major tissues collected and weighed. 2 mL milliQ water was added to each liver and gut samples which were then homogenized and 500 μ L samples taken for analysis. All tissues were then dissolved by incubation with 1 (lymph node) or 2 mL (all other tissues) SOLVABLE (Perkin Elmer) overnight in a 60 °C oven. Samples were then bleached by addition of 30% hydrogen peroxide and Ultima Gold (Perkin Elmer) scintillation cocktail added. Samples were then incubated at 4 °C for 2 days before being analysed on a Tri-Carb 4910TR 110V liquid scintillation counter. All collected values were background corrected by subtraction of values obtained from tissues of a saline injected animal and then normalized to injected dose from dose samples run on the same day in triplicate. These were then normalized per gram of tissue to obtain percentage injected dose per gram of tissue (%ID/g).

Results

[0310] One hour after POPC/POPG/0.5%PEG/PI₃₃₋₆₃/calcitriol or EPC/EPG/PI₃₃₋₆₃/calcitriol liposome administration, the skin injection site contained the highest radiation of any organ. Radiation at 72 hours was at background levels across tissues, consistent with the metabolism of administered [³H]Calcitriol by this time. Some residual radiation in the gut at 72 hours in mice that received EPC liposomes reflects slower fecal excretion in this group.

[0311] The radiation was significantly higher at 1 hour than at 72 hours at injection site, liver, kidney, and axillary lymph nodes, and gut, identifying calcitriol biodistribution to these organs. Comparing the two formulations in the first 1 hour, radiation was significantly higher at the injection site and lower in the liver, spleen and kidney after POPC/POPG/0.5%PEG/PI₃₃₋₆₃/calcitriol than EPC/EPG/PI₃₃₋₆₃/calcitriol liposomes, indicating that calcitriol was distributed systemically to a greater extent and less was retained at the injection site after injection of EPC/EPG than POPC/POPG/PEG liposomes. Radiation was equivalent in inguinal or axillary

draining lymph nodes 1 hour after injection of either POPC/POPG/PEG or EPC/EPG liposome formulations.

Conclusions

[0312] These calcitriol biodistribution data support the conclusion that the calcitriol payload of EPC/EPG liposomes is less stably retained than in POPC/POPG/PEG liposomes, permitting greater systemic spread within the first hour after subcutaneous injection.

Sequences of the Disclosure

SEQ ID NO:	Sequence
1	GIAGFKGEQGPKGEB
2	EAEDLQVGQVELGGGPGAGSLQPLALEGSLQ
3	ISQAVHAAHAEINEAGR
4	VVLLVATEGXVRVNSAYQDK
5	MVVTRTEKDSYVVAFSDEMVPCTVT
6	GSALRPSTSRSLYASSPGGVYATRSSAVRLRSSVPGVRLQLQDSVD
7	GSALRPSTSRSLYASSPGGVYATXSSAVRLRSSVPGVRLQLQDSVD
8	YSLGSALRPSTSRSLYASSPGGVYATXSSAVRLRSSVPGVRLQLQDSVDFSL
9	PRWDGERLYQEARKIVGAMVQIITYRDYLPL
10	RKIVGAMVQIITY
11	DAGWLADQTVXYPIHTPREGCYGDKDEFPGVXTYGIXDTNETYDV
12	TLLWVFVTLXVITAAVTV
13	IVFHYXAISTXYTLDF
14	STXYTLDFDXAQXACLQ
15	DAGWLADQTVXYPIHT
16	DEFPGVXTYGIXDTNETYDV
17	SAGWLADXSVPXYPIK
18	GVVFHYXPGPTXYSLTF
19	GYEQCDAGWLXDQTVXYPIV
20	PGVXTYGVPSTETVDVY
21	DVYCFVDXLEGEVFFA
22	EVFFATXLEQFTFQE
23	KCYAGWLADGSLXYPIV
24	NSPFCLEXTPLGSPDPA
25	GGMGCSSPPCECHQEE
26	ECHQEEDFRVTCKDIQRIPS

27	IQRIPSLPPSTQTLKLIET
28	LKLIETHLRTIPSHAFSNL
29	HAFSNLPNISRIYVSIDVT
30	VSIDVTLQQLESHSFYNLSK
31	FYNLSKVTHIEIRNTR
32	RNTRNLTYIDPDALKE
33	PDALKELPLLKFLGIFNTG
34	GIFNTGLKMFDPDLTKVYST
35	TKVYSTDIFFILEITDNPY
36	ITDNPYMTSIPVNAFQGLC
37	FQGLCNETLTLKLYN
38	KLYNNGFTSVQGYAFN
39	YAFNGTKLDAVYLNKN
40	LNKNKYLTVIDKDAFG
41	DKDAFGGVYSGPSLLD
42	GPSLLDVSQTSVTALP
43	SVTALPSKGLEHLKEL
44	HLKELIARNTWTLKKL
45	WTLKKLPLSLSFLHLTRADL
46	LTRADLSYPHCCAFKNQKK
47	FKNQKKIRGILESLMCNESS
48	CNESSMQSLRQRKSVNALNS
49	VNALNSPLHQEYEENLGDSI
50	NLGDSIVGYKEKSKFQDTHN
51	FQDTHNNAHYVVFEEQEDE
52	EEQEDEIIGFGQELKNPQEE
53	KNPQEETLQAFDSHYDYTIC
54	YDYTICGDSMDVCTPKSDE
55	TPKSDEFNPCEDIMGYKFLR
56	VSLISXXGDMSSNPA
57	FDXYXLNYSLPTGQW
58	PDGFXLSWTADEGVF
59	QGQYELXVDLXDHGE
60	GQVELGGGNAVEVLK
61	GQVELGGGTPIESHQ

62	GQVELGGGSSPETLI
63	GDLQTLWSRMD
64	LQTLALWSRMD
65	LQTLALNAARD
66	YASSPGGVYATXSSAVRLRSSVPGVRLQLQDSVD
67	VYATXSSAVRLRSSVPGVRLQLQDSVD
68	MSTRSVSSSSYRRMFGGPGTASRPSSRSYVTTSTRTYSLGSALRPSTSRSLYASSPGGVYATRS SAVRLRSSVPGVRLQLQDSVDFSLADAINTEFKNTRTNEKVELQELNDRFANYIDKVRFLQEQN KILLAELEQLKGQGSRLGDLYEEEMRELRRQVDQLTNDKARVEVERDNLAEDIMRLREKLQ EEMLQREEAENTLQSFQDQVDNASLARLDLERKVESLQEEIAFLKKLHEEEIQELQAQIQEQH VQIDVDVSKPDLTAALRDVRQQYESVAAKNLQEAEEWYKSKFADLSEAANRNNDALRQAKQ ESTEYRRQVQSLTCEVDALKGTNESLERQMREMEENFAVEAANYQDTIGRLQDEIQNMKEEM ARHLREYQDLLNVKMALDIEIATYRKLEGEESRISLPLPNFSSLNLRETNLDSLPLVDTHSKRT LLIKTVETRDGQVINETSQHDDLE
69	ATEGRVRVNSAYQDK
70	VTQEIVTERSVSSRQAQ
71	GVYATRSSAVRLR

CLAIMS

1. A lipid delivery vehicle comprising a nuclear factor- κ B (NF- κ B) inhibitor and a peptide antigen, wherein the lipid delivery vehicle comprises 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG).
2. The lipid delivery vehicle of claim 1, further comprising a PEGylated lipid selected from the group consisting of PEG-modified phosphatidylethanolamines, PEG-modified phosphatidic acids, PEG-modified ceramides, PEG-modified dialkylamines, PEG-modified diacylglycerols, and PEG-modified dialkylglycerols, PEG-c-DOMG, PEG-DMG, PEG-DLPE, PEG-DMPE, PEG-DPPC, PEG-DSPE and combinations thereof.
3. The lipid delivery vehicle of claim 2, wherein the lipid delivery vehicle comprises about 85 mol % to 95 mol % of POPC, about 5 mol % to 15 mol % of POPG and about 0.1 mol % to about 5 mol % of the PEGylated lipid.
4. The lipid delivery vehicle of claim 3, wherein the lipid delivery vehicle has a mean diameter of between about 60 nm to about 180 nm.
5. The lipid delivery vehicle of claim 4, wherein the lipid delivery vehicle has a negative surface charge that is less than about -30 mV.
6. The lipid delivery vehicle of any one of claims 1 to 5, wherein the peptide antigen is an autoantigen derived from the protein selected from the group consisting of Ro, collagen type II (CII), proinsulin (PI), insulin, ovalbumin, tenascin C, chromogranin, aggrecan, islet antigen 2 (IA2), glutamic acid decarboxylase 65-kilodalton isoform (GAD65), glycoprotein (gp70), nuclear antigens, lupus autoantigen, Smith, La, U1-RNP, fibrillin, histones, ribosomal proteins, pyruvate dehydrogenase dihydrolipoamide acetyltransferase (PCD-E2), hair follicle antigens, human tropomyosin isoform 5 (hTM5), human cartilage gp 39 (HCgp39) and gp130-RAPS, dnaJp1, citrullinated proteins, citrullinated peptides, citrullinated type II collagen, citrullinated vimentin, citrullinated fibrinogen, citrullinated aggrecan, citrullinated tenascin C, myelin basic protein, proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG), thyroid stimulating factor receptor (TSH-R), acetylcholine receptor (AChR), gliadin, histones, PLP, glucose-6-phosphate isomerase, thyroglobulin, various tRNA synthetases, proteinase-3, human desmoglein 2 (hDSG2) and myeloperoxidase (MPO).
7. The lipid delivery vehicle of claim 6, wherein the peptide antigen is an autoantigen derived from the protein selected from the group consisting of collagen, proinsulin, ovalbumin, insulin, aggrecan, Ro, vimentin, thyroid stimulating factor receptor (TSH-R), myeloperoxidase

(MPO), tenascin C, human desmoglein 2 (hDSG2), citrullinated vimentin, citrullinated aggrecan, and citrullinated tenascin C.

8. The lipid delivery vehicle of claim 7, wherein the peptide antigen is an autoantigen derived from the protein selected from the group consisting of collagen, proinsulin, ovalbumin, insulin, aggrecan, Ro, vimentin, human desmoglein 2 (hDSG2), citrullinated vimentin, and citrullinated aggrecan.

9. The lipid delivery vehicle of claim 8, wherein the peptide antigen is an autoantigen derived from proinsulin or insulin.

10. The lipid delivery vehicle of claim 8, wherein the peptide antigen is an autoantigen derived from aggrecan or citrullinated aggrecan.

11. The lipid delivery vehicle of claim 8, wherein the peptide antigen is an autoantigen derived from vimentin or citrullinated vimentin.

12. The lipid delivery vehicle of claim 8, wherein the peptide antigen comprises, consists of or consists essentially of an amino acid sequence set forth in any one of SEQ ID NOs: 1-71, or a variant thereof, wherein the variant shares at least 80% sequence identity with the amino acid sequence.

13. The lipid delivery vehicle of claim 12, wherein the peptide antigen comprises, consists of or consists essentially of an amino acid sequence set forth in any one of SEQ ID NOs: 4, 11-24, or 69, or a variant thereof, wherein the variant shares at least 80% sequence identity with the amino acid sequence.

14. The lipid delivery vehicle of claim 12, wherein the peptide antigen comprises, consists of or consists essentially of an amino acid sequence set forth in any one of SEQ ID NOs: 6-8, 66-68 or 71, or a variant thereof, wherein the variant shares at least 80% sequence identity with the amino acid sequence set forth in the SEQ ID.

15. The lipid delivery vehicle of claim 12, wherein the peptide antigen comprises, consists of or consists essentially of an amino acid sequence set forth in any one of SEQ ID NOs: 2, or 60-65, or a variant thereof, wherein the variant shares at least 80% sequence identity with the amino acid sequence set forth in the SEQ ID.

16. The lipid delivery vehicle of claim 12, wherein the peptide antigen comprises, consists of or consists essentially of an amino acid sequence set forth in SEQ ID NO: 2, or variant thereof, wherein the variant shares at least 80% sequence identity with the amino acid sequence of SEQ ID NO. 2.

17. The lipid delivery vehicle of claim 6, wherein the NF- κ B inhibitor is selected from the group consisting of calcitriol, rapamycin, leflunomide, teriflunomide, bevacizumab, bithionol, bortezomib, doxorubicin hydrochloride, cantharidin, carfilzomib, chromomycin daunorubicinum, digitoxin, ectinascidin, emetine, erlotinib hydrochloride, gemcitabine, irinotecan, quinacrine dihydrochloride, fluorosalan, manidipine hydrochloride, narasin, lestaurtinib, ouabain, pemetrexed disodium, sorafenib tosylate, sunitinib malate, tioconazole, topotecan, tribromsalan, triclabendazolum, zafirlukast, withaferin A, quecertin, curcumin, BAY 11-7085 and BAY 11-7082, and/or active metabolites and synthetic analogs thereof, and combinations thereof.

18. The lipid delivery vehicle of claim 17, wherein the NF- κ B inhibitor teriflunomide, or leflunomide, and/or an active metabolite or a synthetic analog thereof.

19. The lipid delivery vehicle of claim 6, wherein the NF- κ B inhibitor is a Vitamin D, an active metabolite of Vitamin D, a previtamin D, or a synthetic vitamin D analog.

20. The lipid delivery vehicle of claim 19, wherein the NF- κ B inhibitor is selected from the group consisting of calcitriol, calciferol, ergocalciferol, alfalcidol, doxercalciferol, calcidiol, 22-oxacalcitriol, paricalcitol, tacalcitol, falecalcitriol, eldecalcitol, inecalcitol, seocalcitol, calcipotriene, maxacalcitol, calcifediol, calcipotriol; synthetic analogs thereof and/or side-chain analogs thereof; and combinations thereof.

21. The lipid delivery vehicle of claim 20, wherein the NF- κ B inhibitor is calcitriol, a side-chain analog thereof or a synthetic analog thereof.

22. The lipid delivery vehicle of any one of claims 1 to 3, wherein the peptide antigen is an autoantigen derived from the proinsulin or insulin, and the NF- κ B inhibitor is Vitamin D, an active metabolite of Vitamin D, a previtamin D, or a synthetic vitamin D analog, and wherein the lipid delivery vehicle comprises from about 1 μ g/mL to about 50 μ g/mL of the peptide antigen, and from about 400 ng/mL to about 600 ng/mL of the NF- κ B inhibitor.

23. The lipid delivery vehicle of claim 22, wherein the at least 50% of NF- κ B inhibitor, and at least 50% of the peptide antigen are encapsulated within the lipid delivery vehicle.

24. The lipid delivery vehicle of claim 23, wherein, when incubated in plasma for 15 minutes at 37°C, the at least 50% of encapsulated NF- κ B inhibitor, and at least 50% of the encapsulated peptide antigen are retained within the lipid delivery vehicle.

25. A population of lipid delivery vehicles according to any one of claims 1 to 24, wherein the population has a polydispersity index of less than 0.20.

26. A pharmaceutical composition for subcutaneous delivery comprising the lipid delivery vehicle of any one of claims 22 to 24 and a pharmaceutically acceptable carrier.
27. A method for eliciting a tolerogenic immune response in a subject, comprising administering to the subject the lipid delivery vehicle of any one of claims 1 to 24 to the subject.
28. Use of the lipid delivery vehicle of any one of claims 1 to 24 in the manufacture of a medicament for eliciting a tolerogenic immune response in a subject.
29. A method for treating and/or preventing an autoimmune disease, disorder or condition in a subject, comprising administering to the subject the lipid delivery vehicle of any one of claims 1 to 24 to the subject.
30. Use of the lipid delivery vehicle of any one of claims 1 to 24 in the manufacture of a medicament for treating and/or preventing an autoimmune disease, disorder or condition in a subject.
31. The method of claim 27 or 29, therein the lipid delivery vehicle is administered subcutaneously.
32. The use of claim 28 or 30, wherein the medicament is formulated for subcutaneous administration.

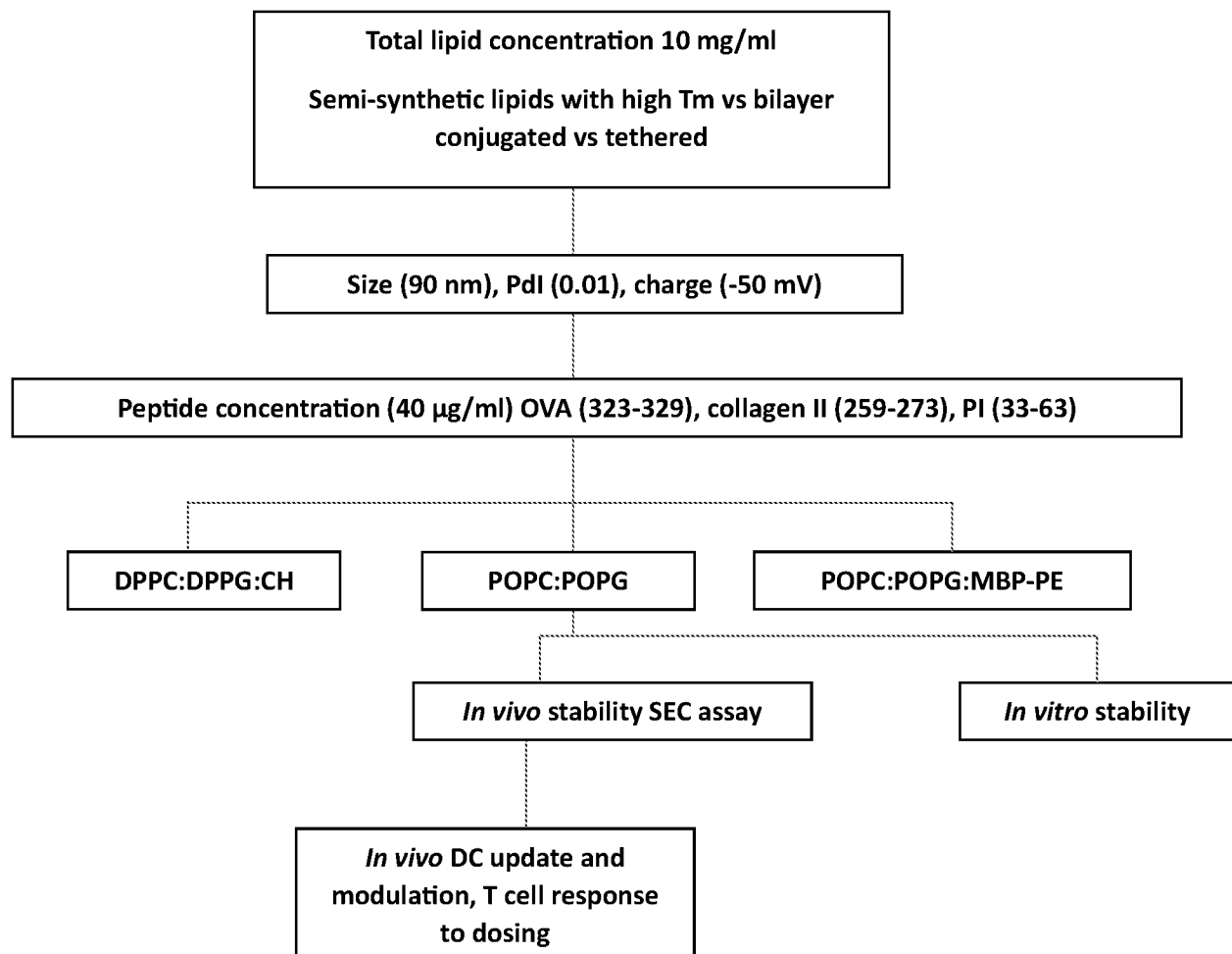
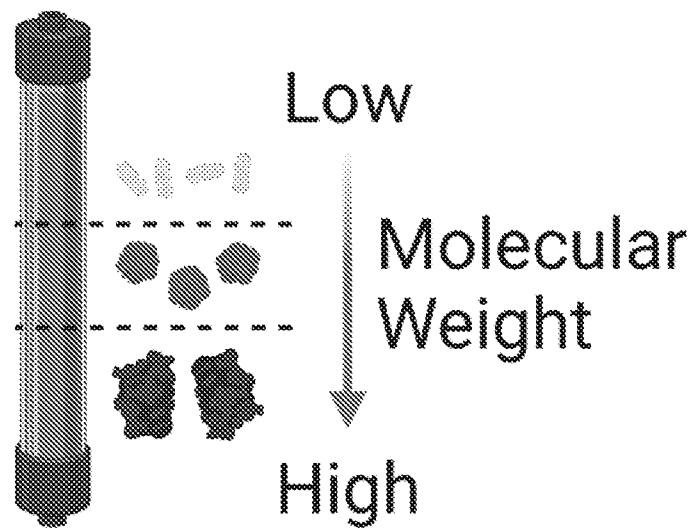


Figure 1

A



B

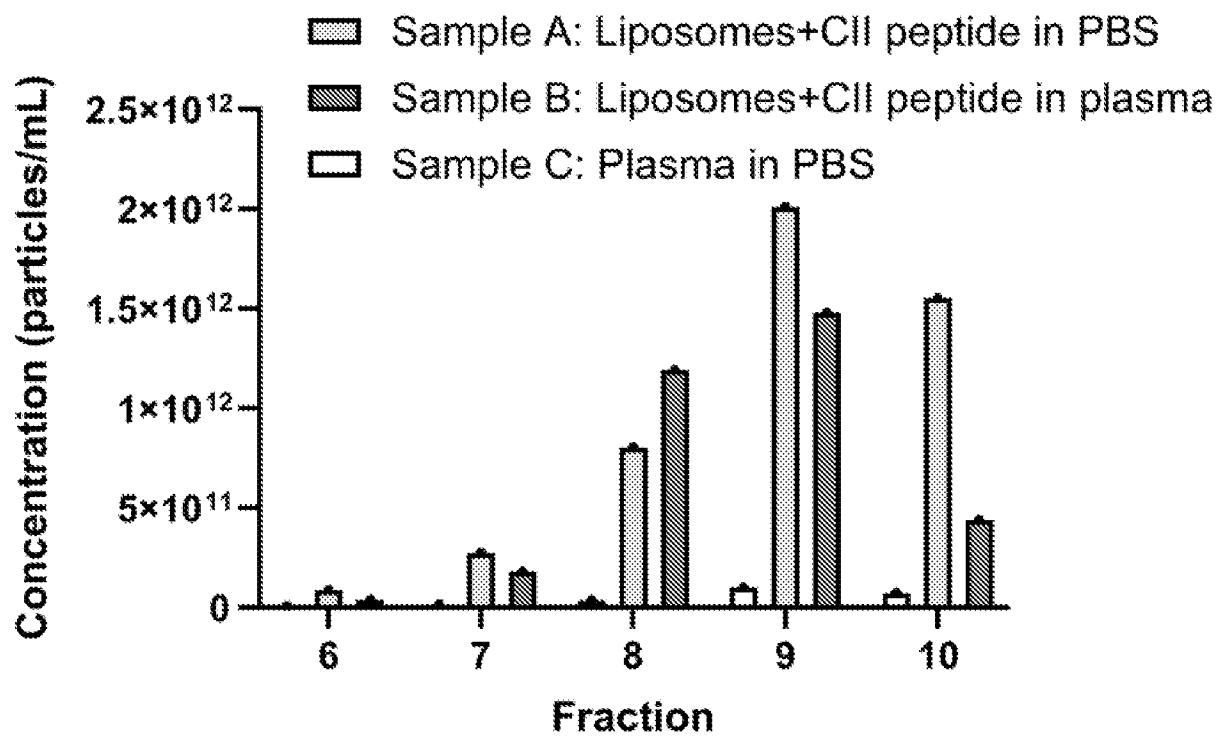
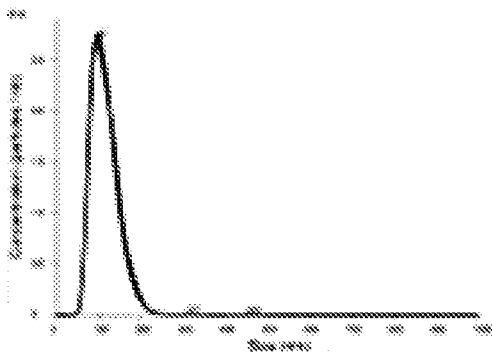


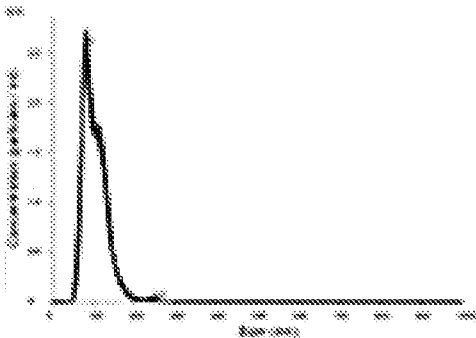
Figure 2

C

Sample_A_SEC_9 - NTA



Sample_B_SEC_9 - NTA



Sample_C_SEC_9 - NTA

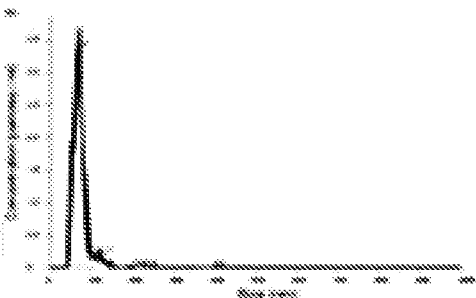


Figure 2 (continued)

D

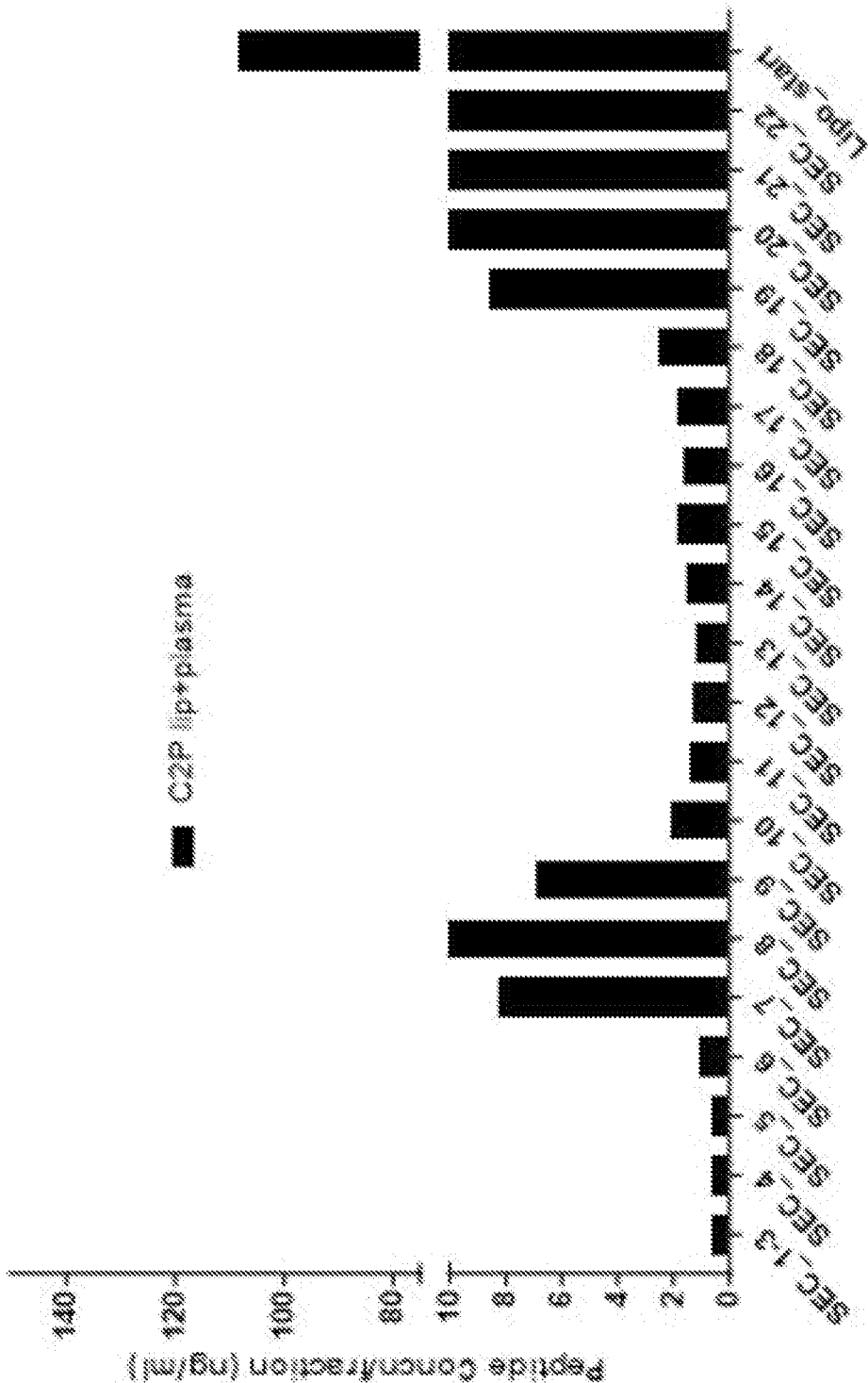


Figure 2 (continued)

E

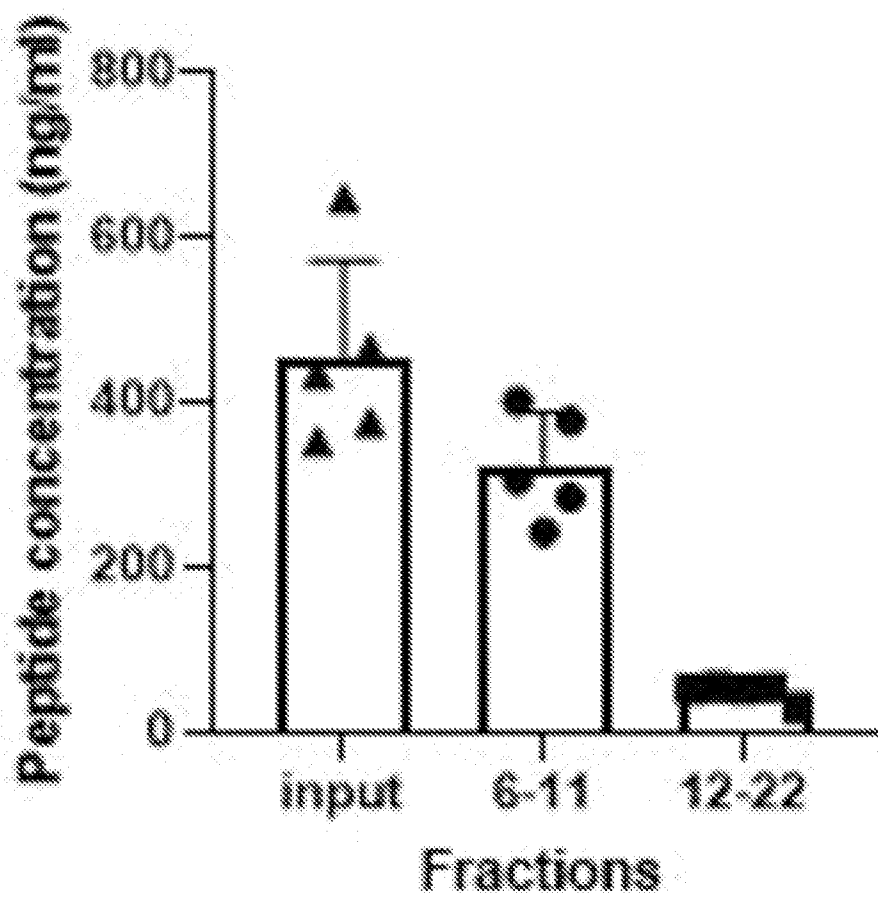
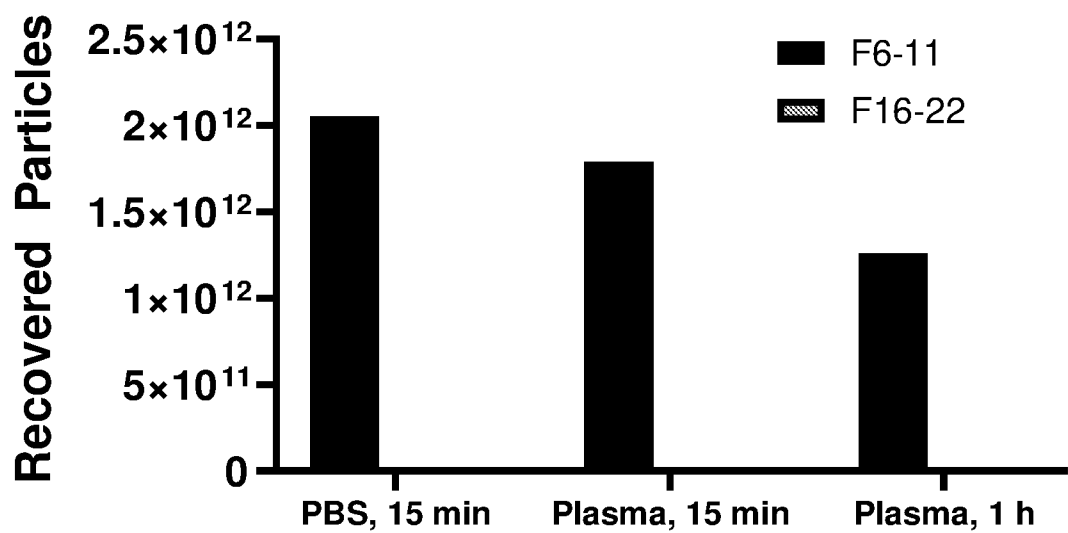


Figure 2 (continued)

A



B

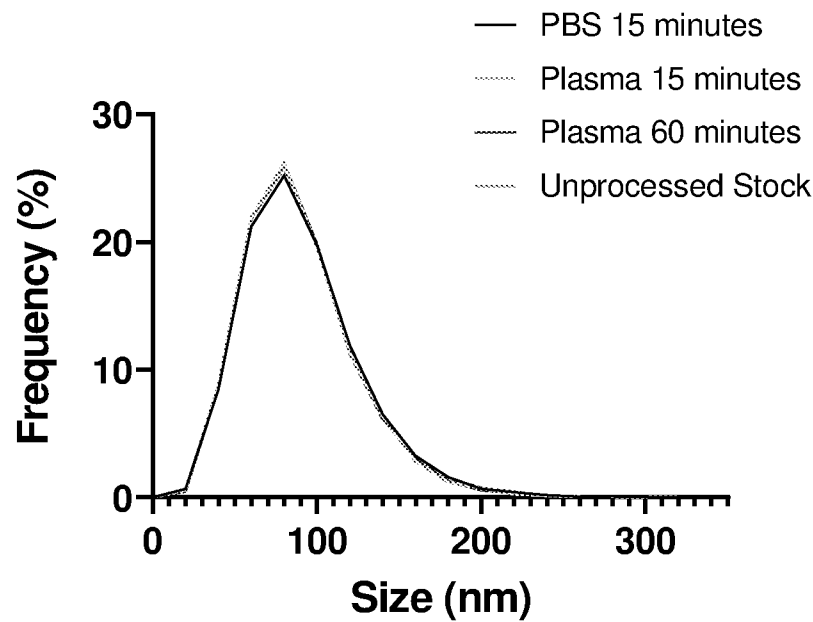


Figure 3

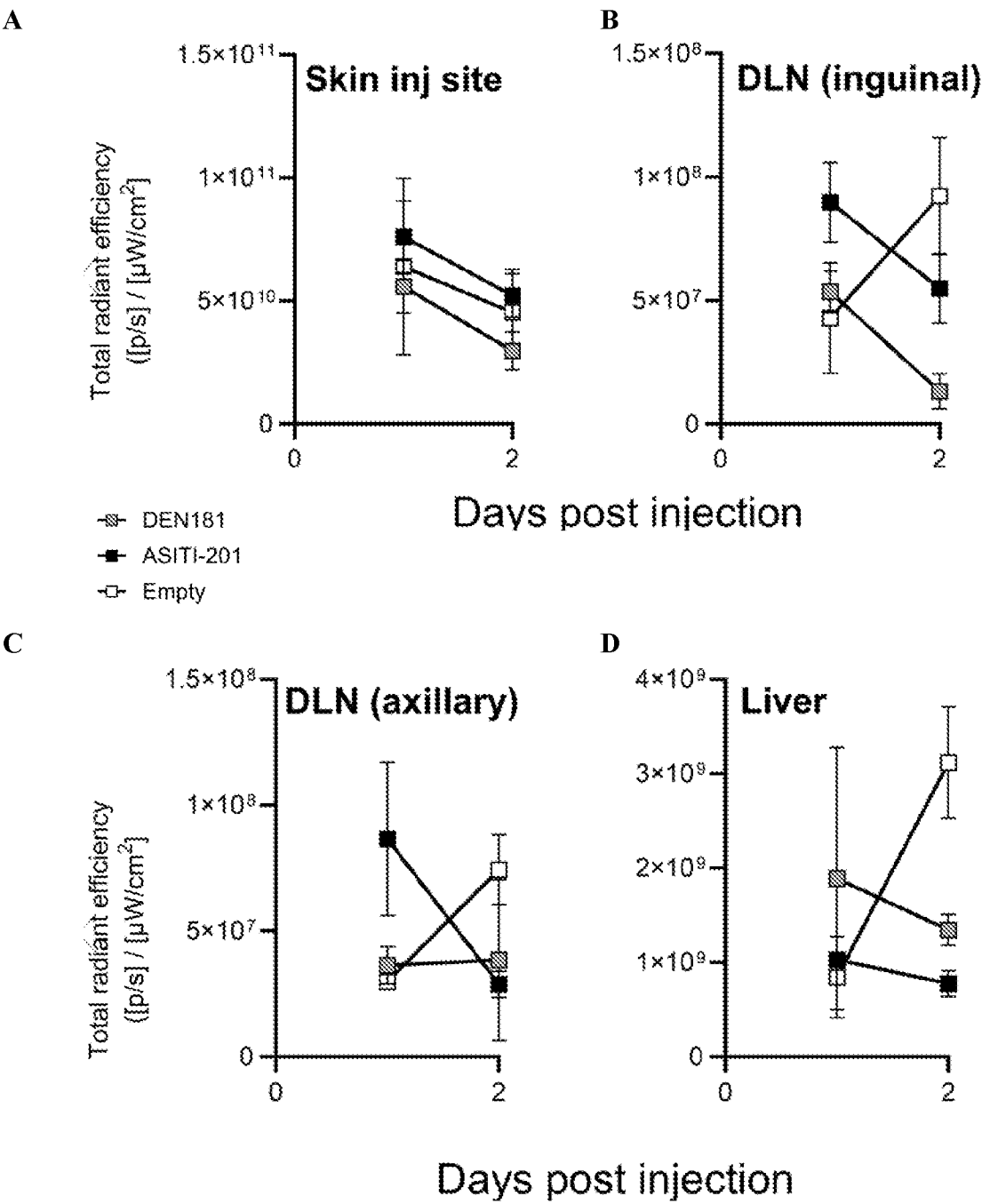
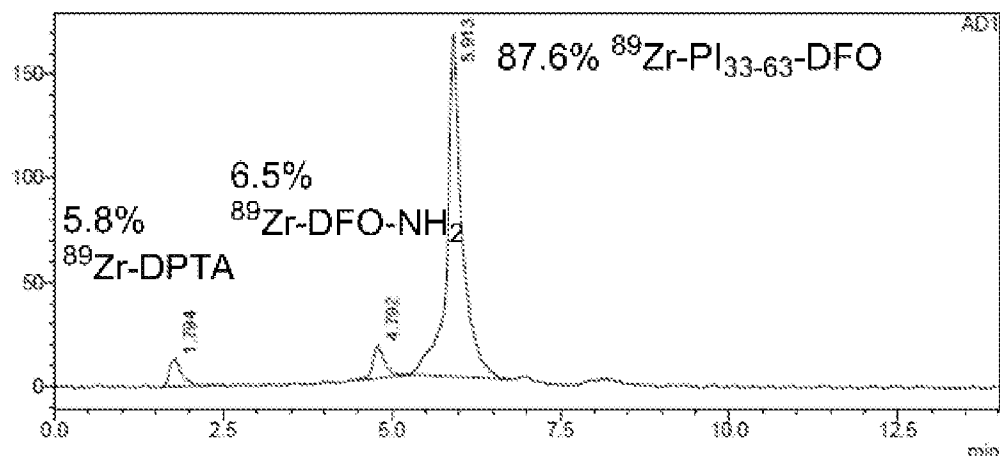


Figure 4

A



B

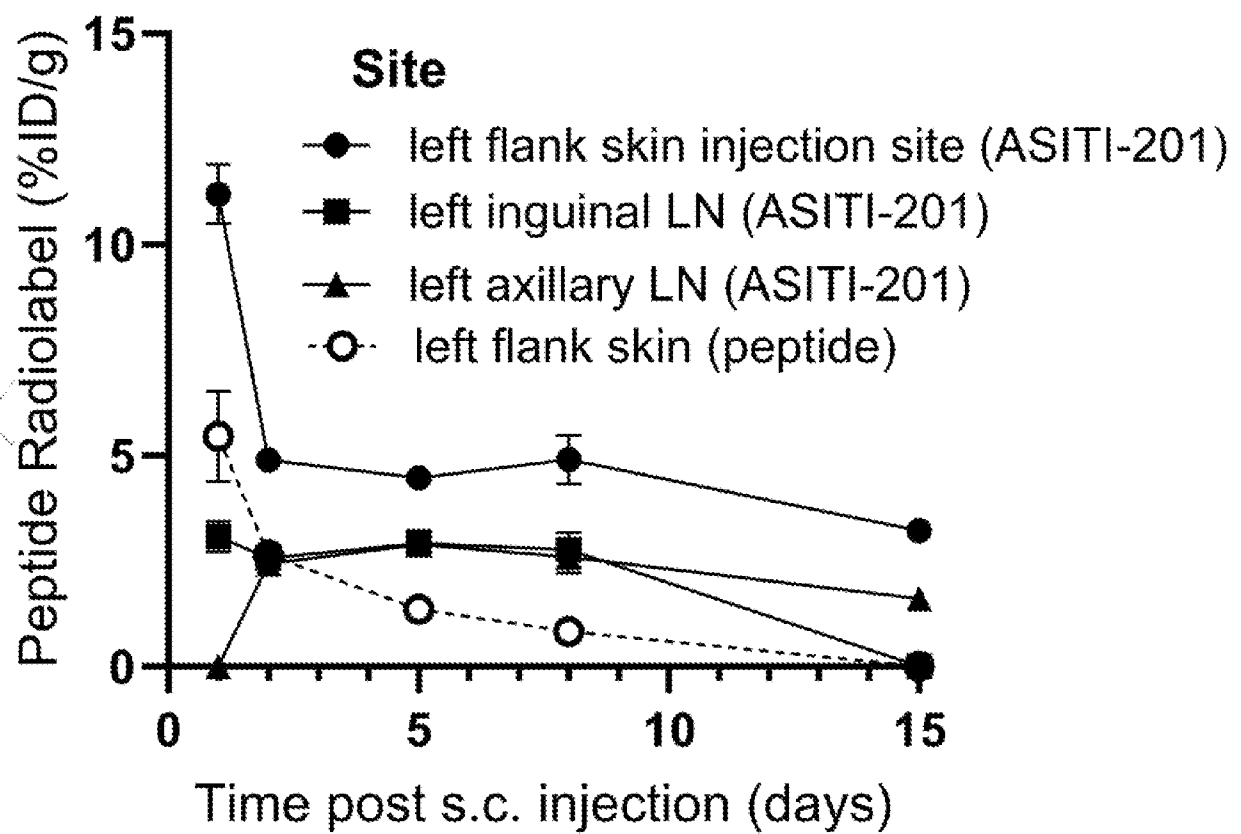


Figure 5

C

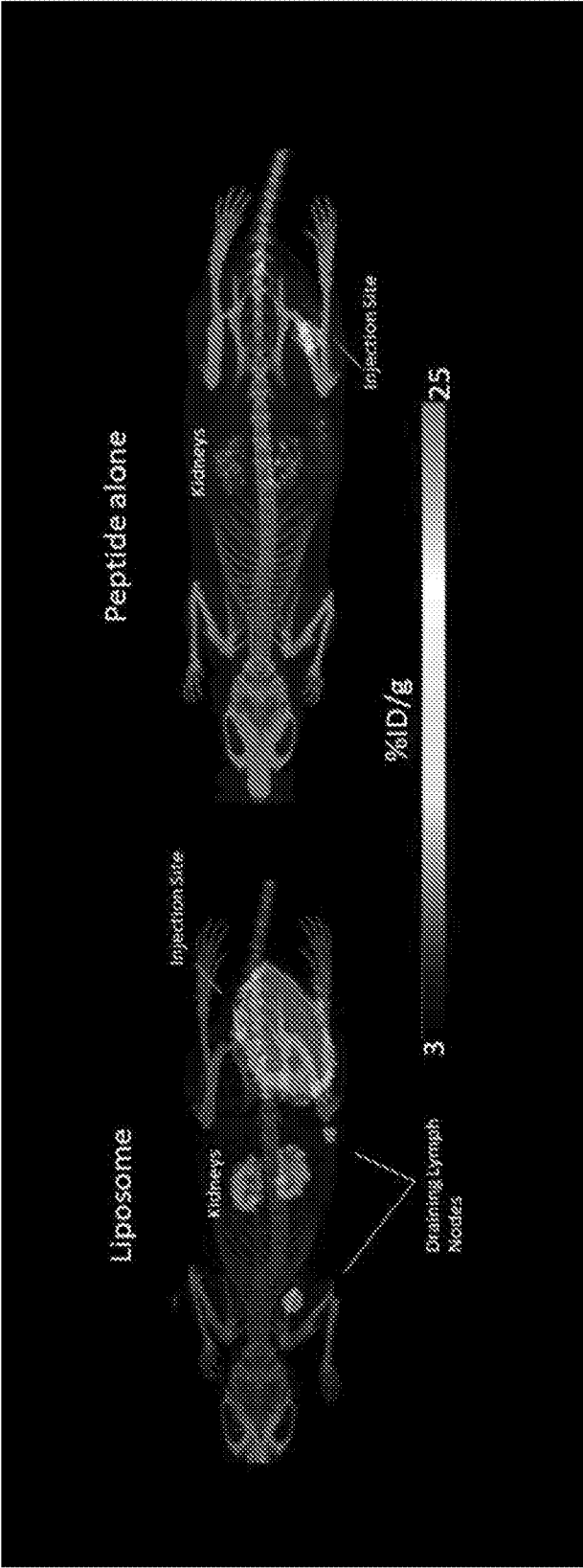
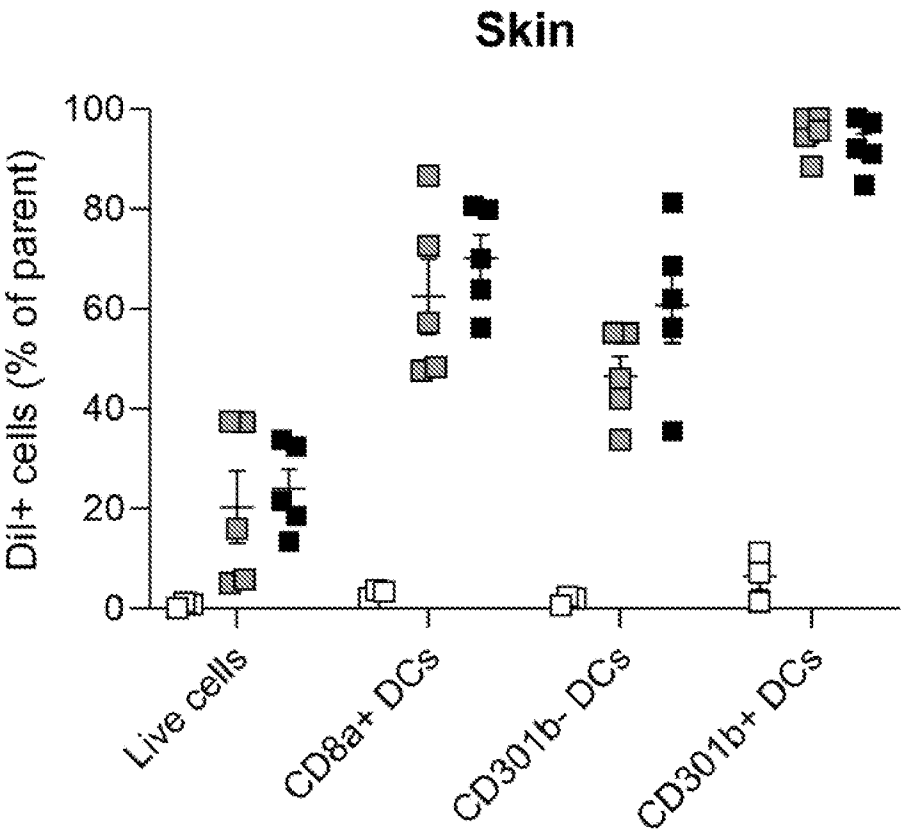


Figure 5 (continued)

A



B

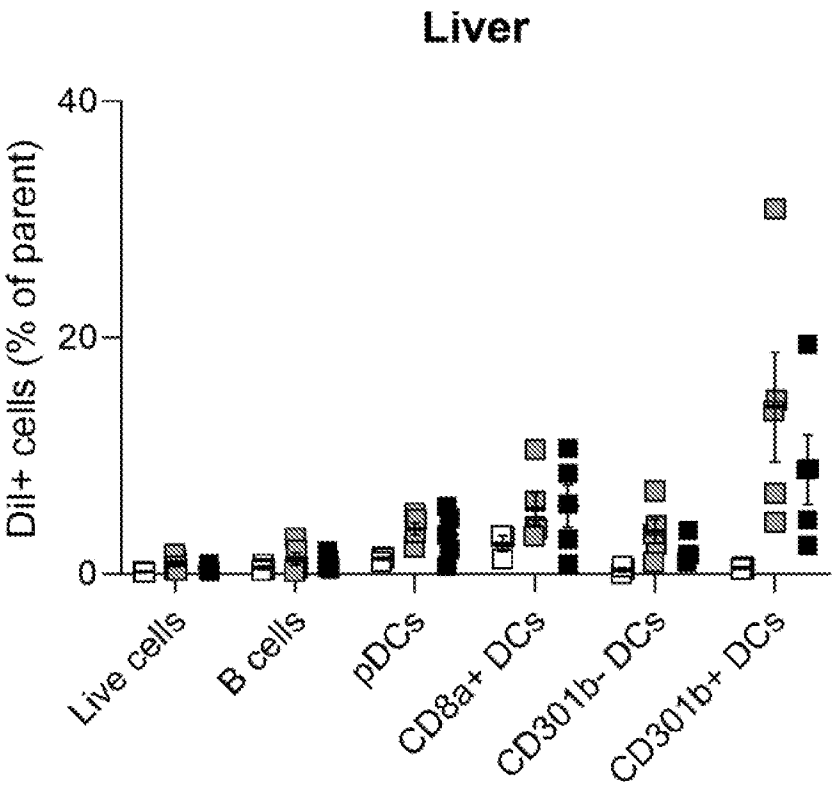
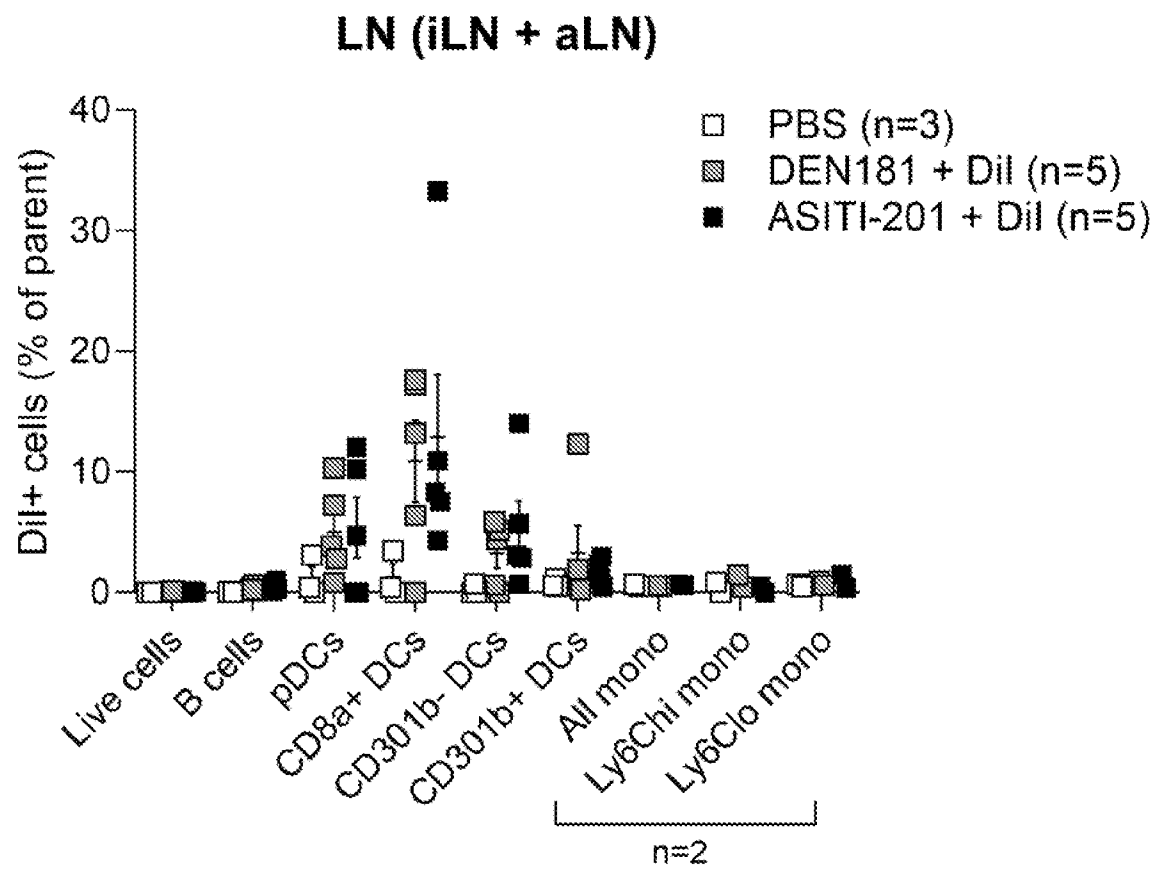


Figure 6

C



D

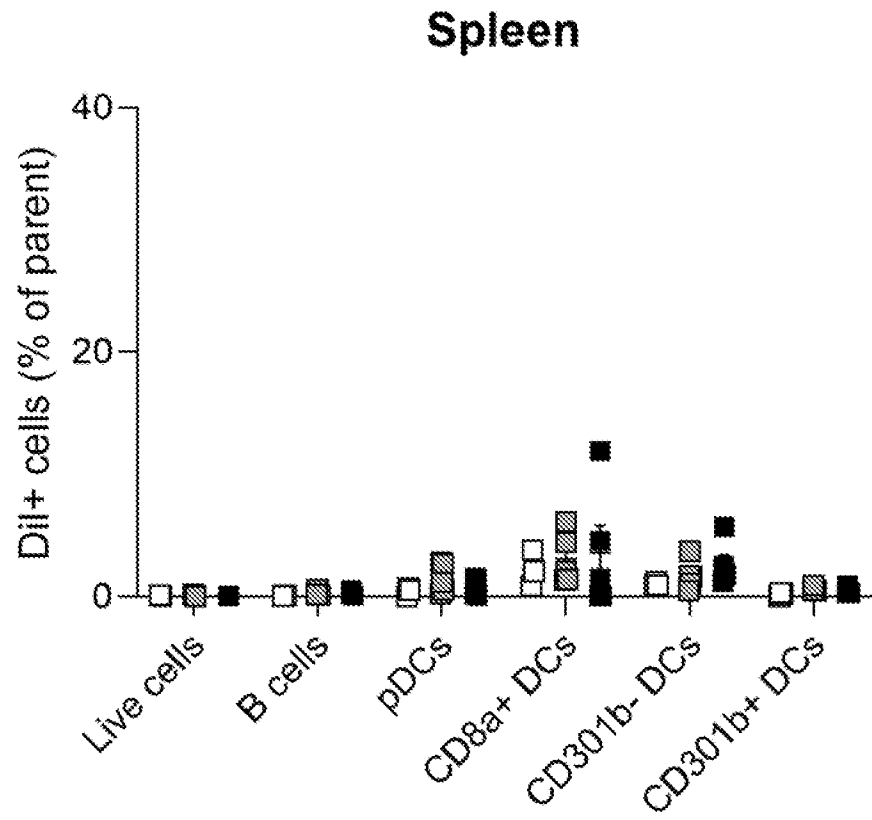


Figure 6 (continued)

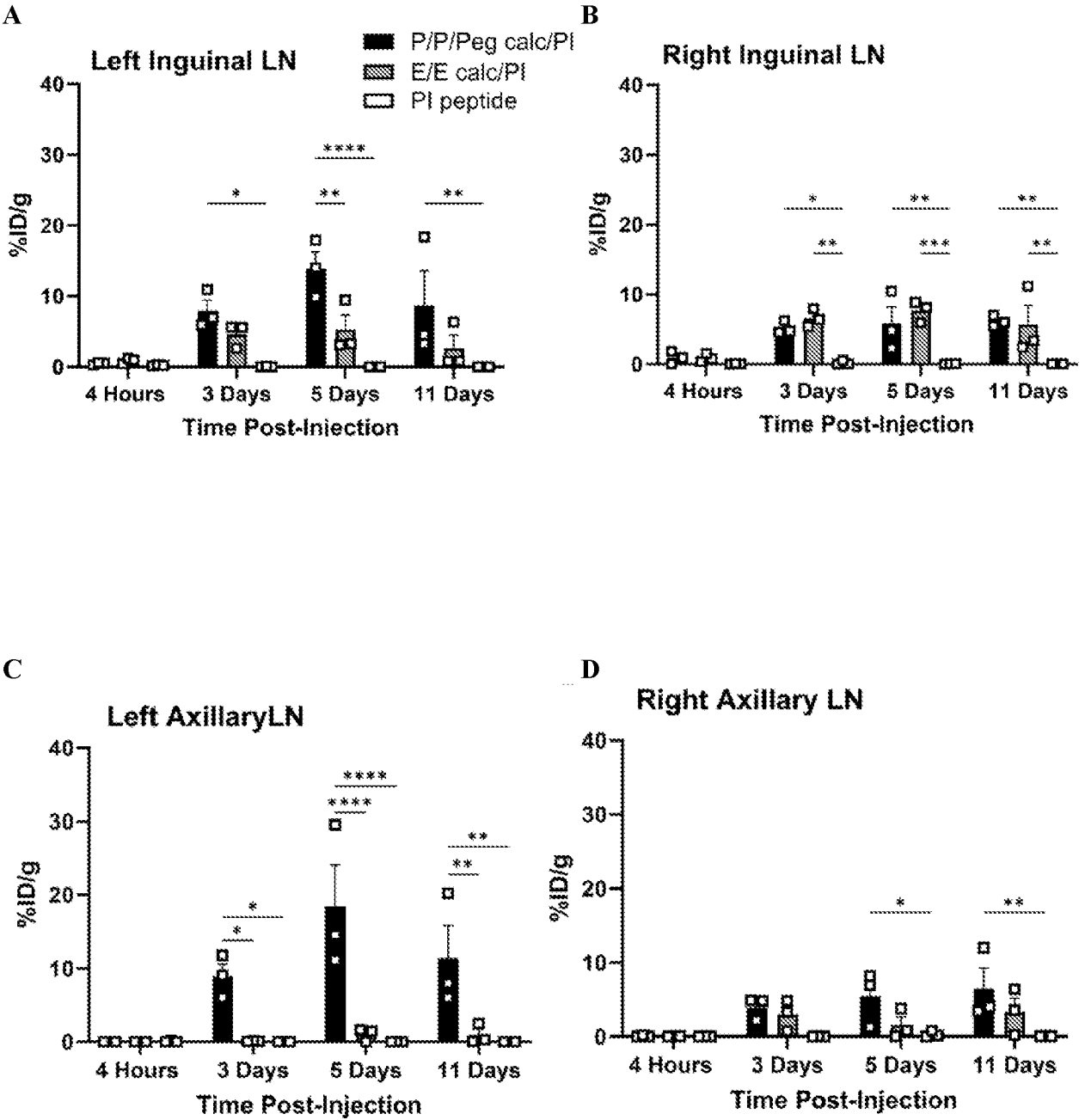
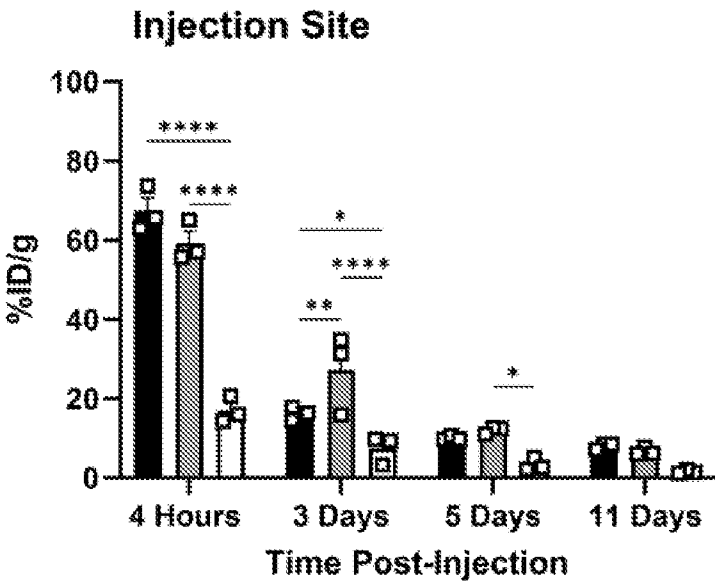


Figure 7

E



F

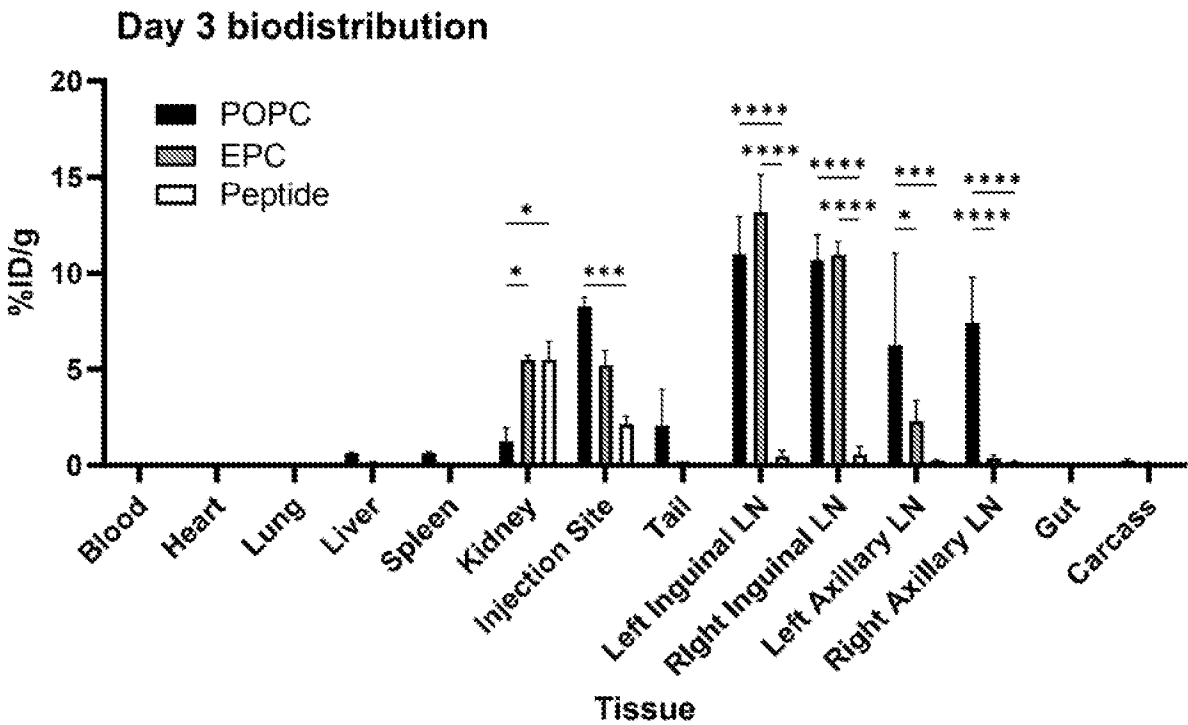


Figure 7 (continued)

G

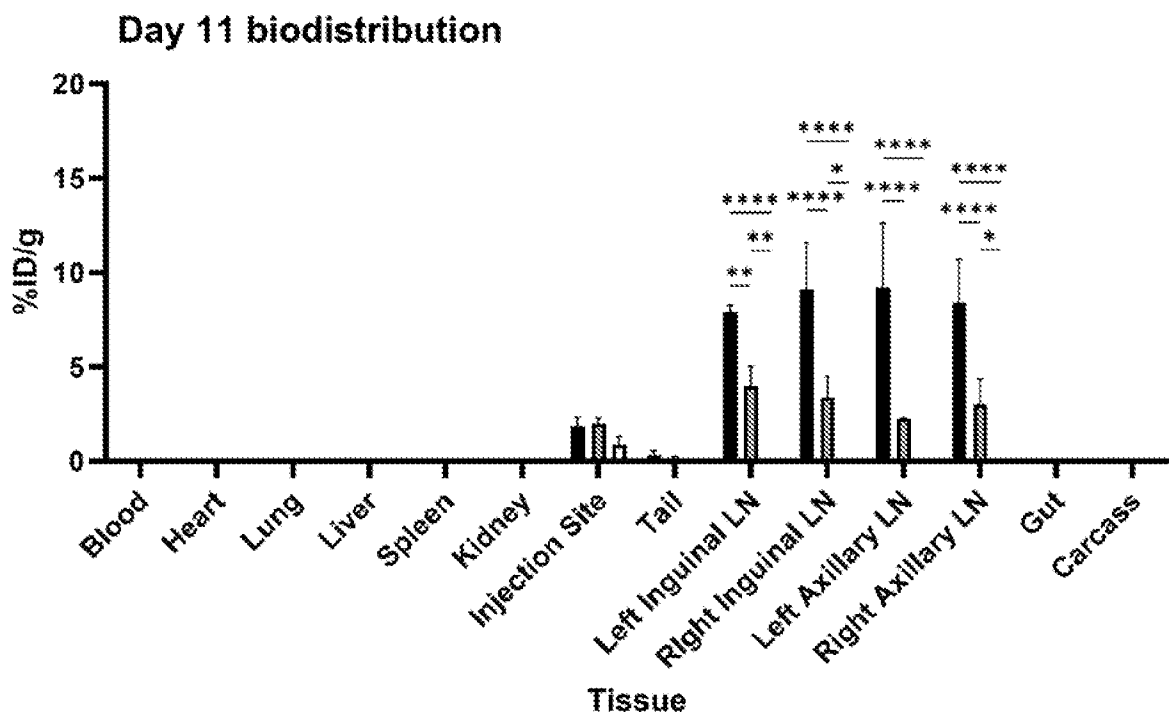
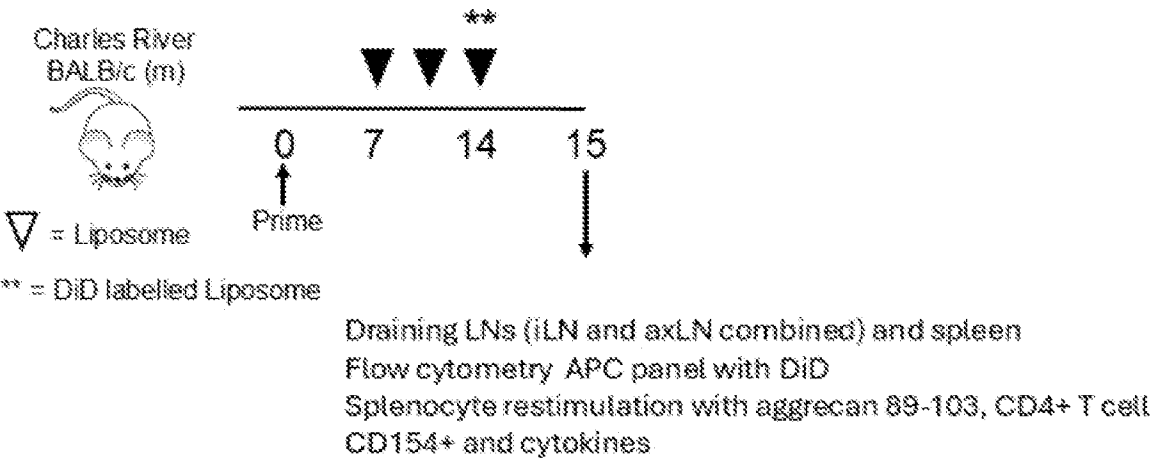


Figure 7 (continued)

A

- 1. POPC/POPG Blank Liposomes (x3 doses) n=3
- 2. EPC/EPG hAgg/calcitriol liposome (x3 doses) n=3
- 3. POPC/POPG hAgg/calcitriol Liposome (x3 doses) n=3



B

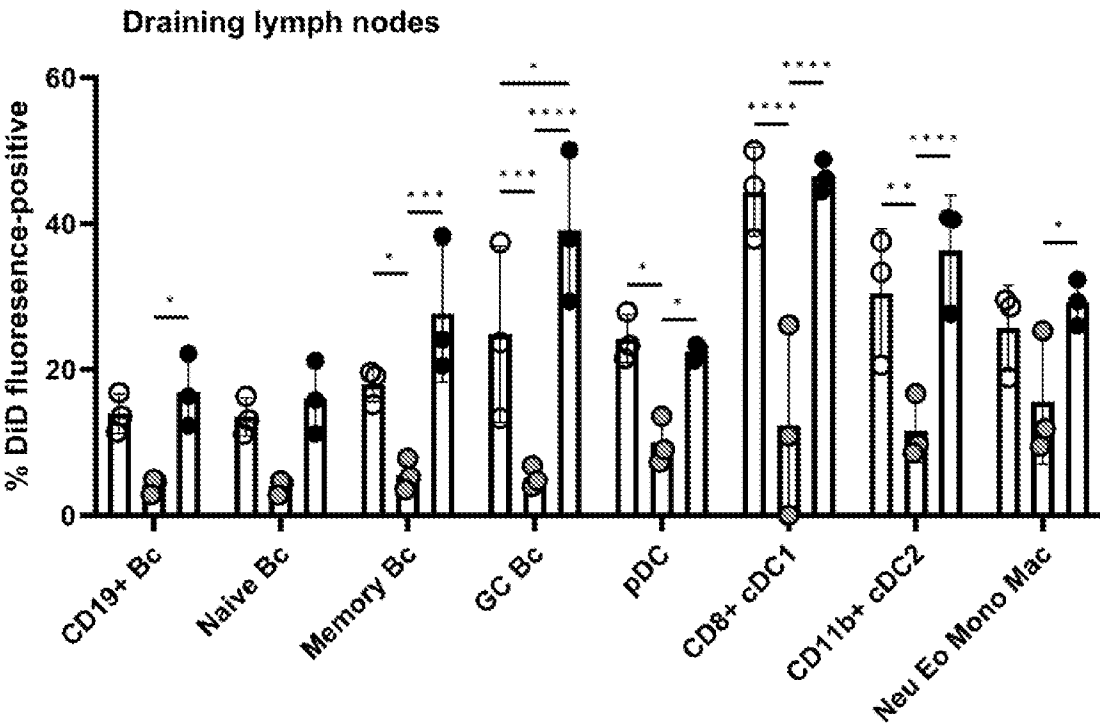


Figure 8 (continued)

C

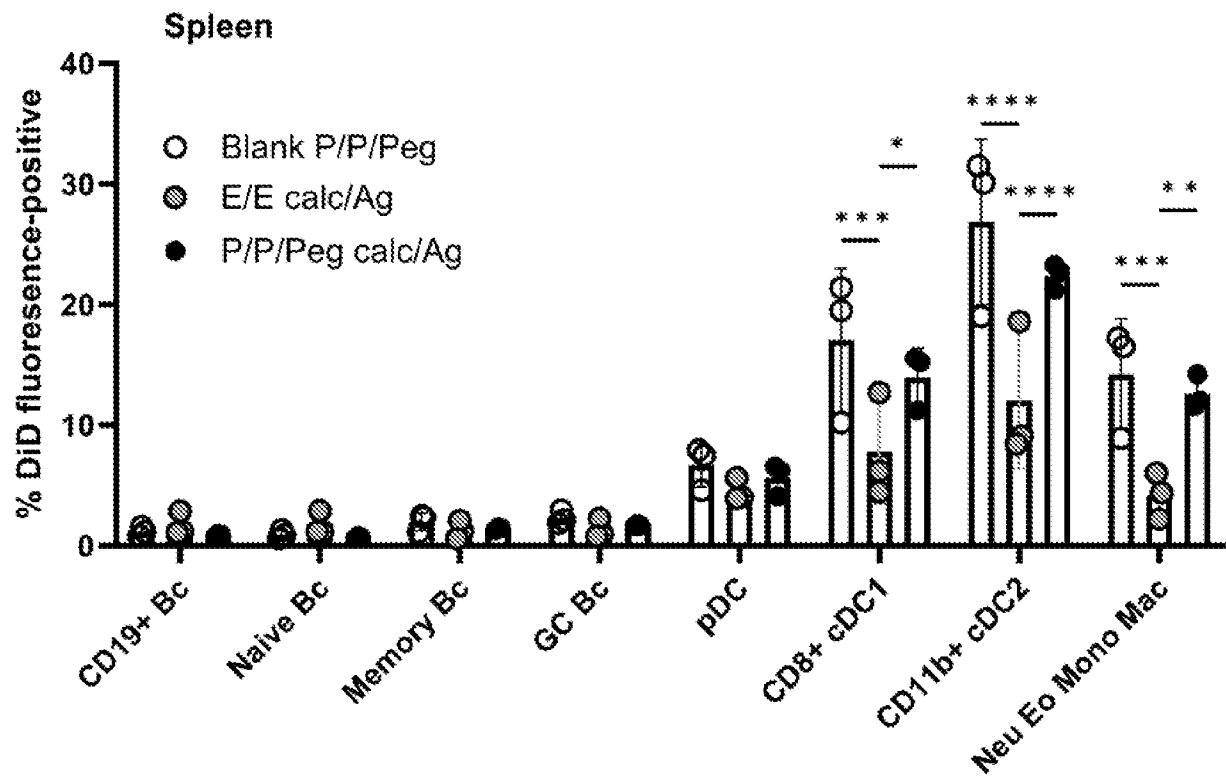
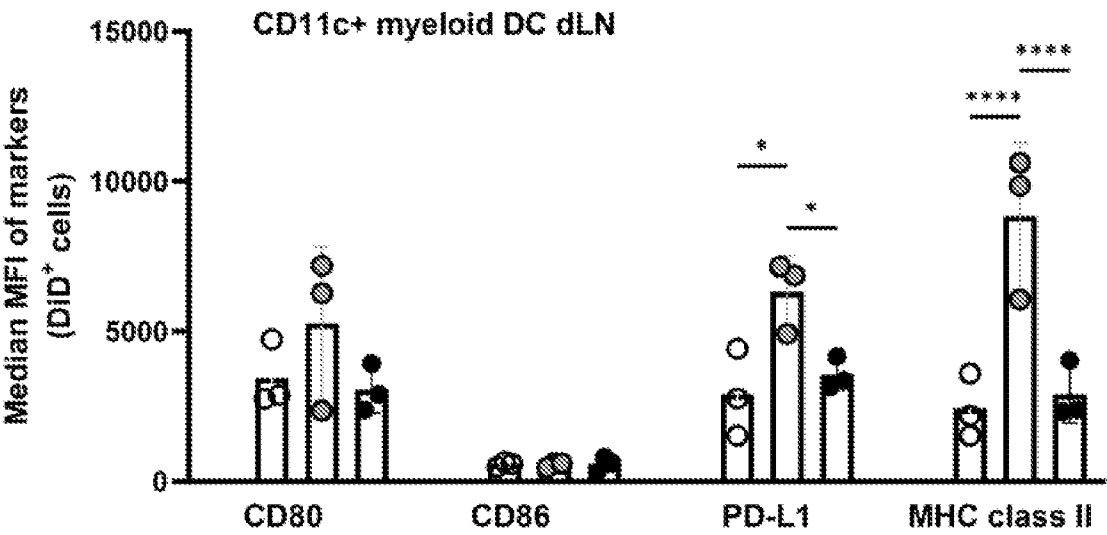
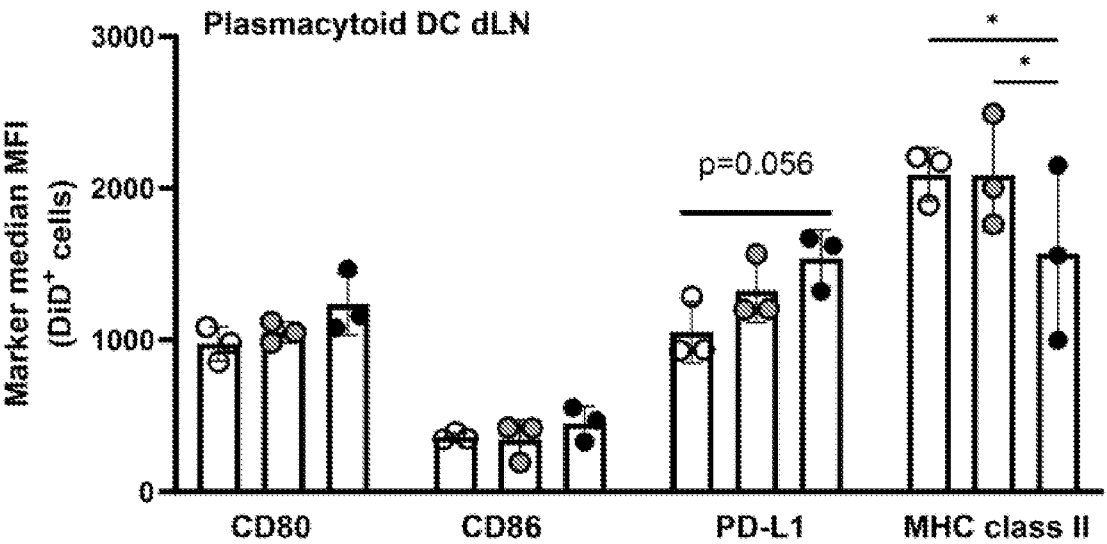


Figure 8 (continued)

A



B



C

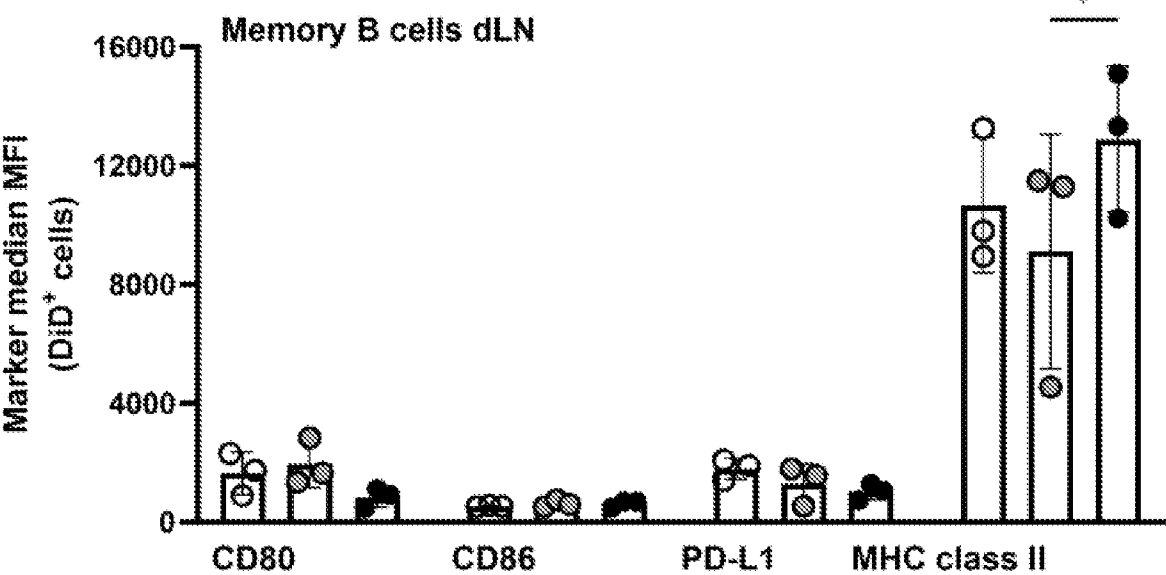
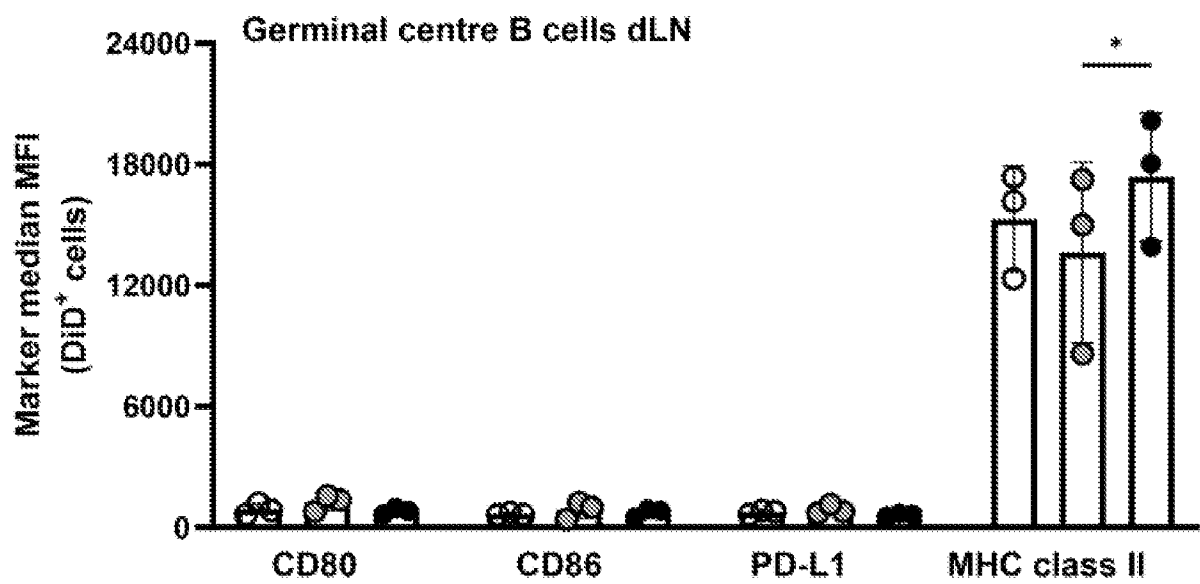


Figure 9

D



E

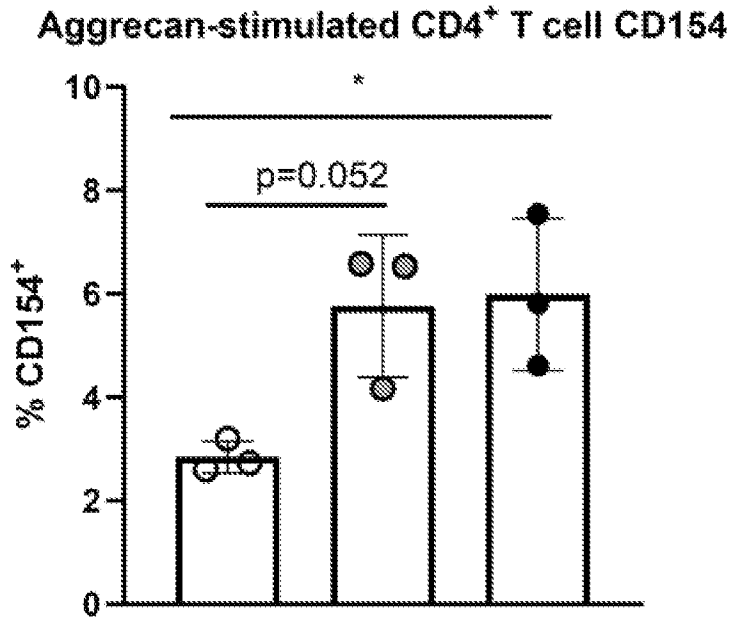
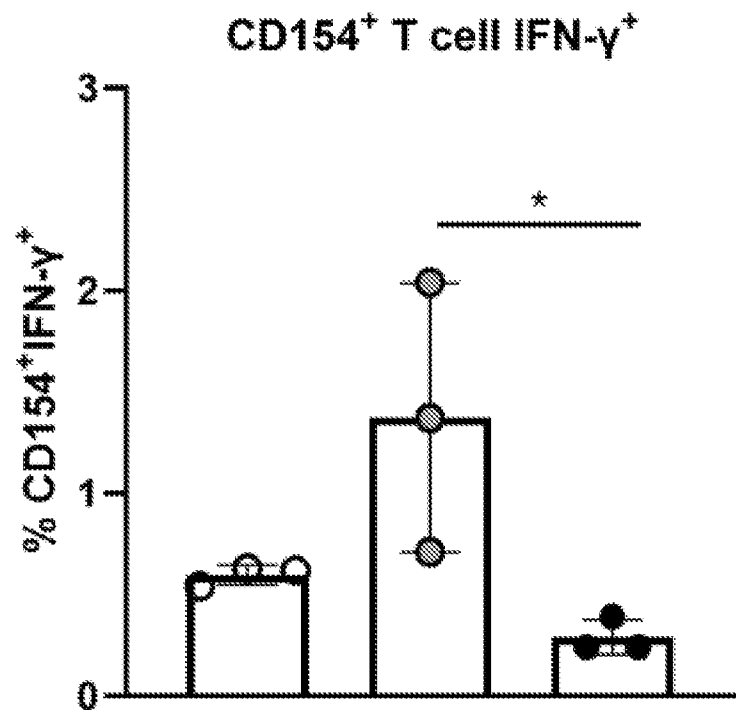


Figure 9 (continued)

F



G

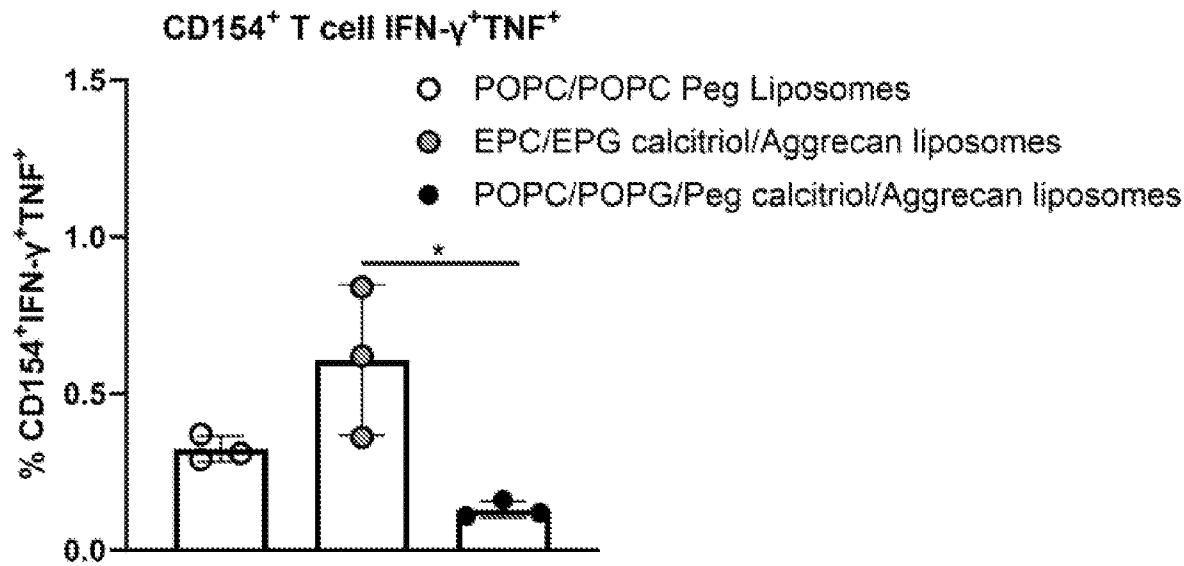


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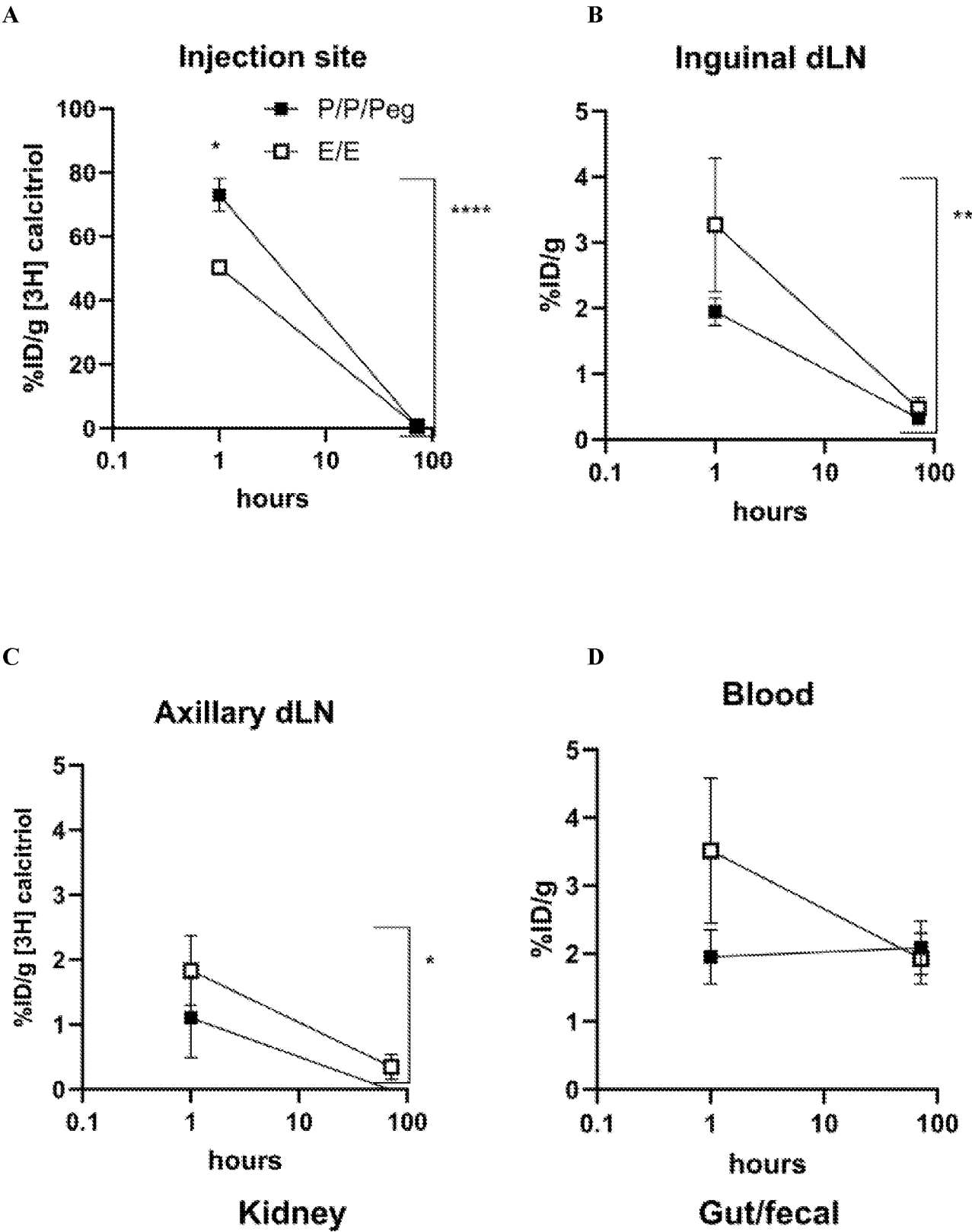


Figure 10

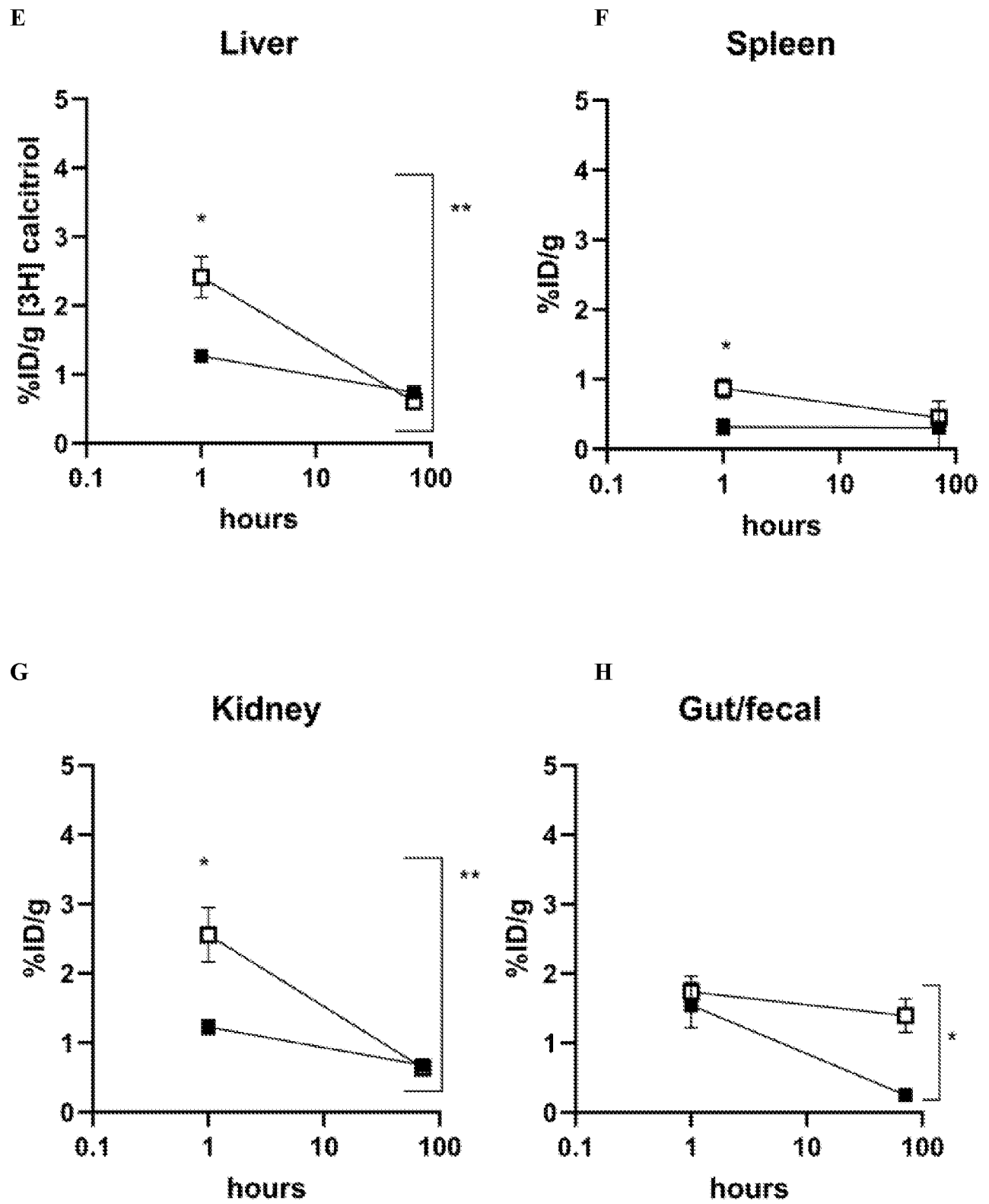


Figure 10 (continued)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2024/051401

A. CLASSIFICATION OF SUBJECT MATTER

A61K 9/1271 (2025.01) A61K 9/00 (2006.01) A61K 9/1272 (2025.01) A61K 9/51 (2006.01) A61K 39/00 (2006.01)
 A61K 47/18 (2017.01) A61K 47/24 (2006.01) A61K 47/28 (2006.01) A61P 37/02 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Google, EPOQUE and STN Search - PATENW, BIOSIS, CAPLUS, EMBASE, MEDLINE, REGISTRY; Keywords – liposome, lipid vehicle, nuclear factor κ B, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine, POPC, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol, POPG, and related terms, 26853-31-6, 185435-28-3, 32222-06-3, A61K9/1271, A61K9/1272, A61K9/51, A61K39/00, A61P37/02

Applicant/Inventor Search (Espacenet, PubMed, AusPAT, Google Patents, IP Australia Internal Databases): Applicant – THE UNIVERSITY OF QUEENSLAND; Inventor(s) – TALEKAR, Meghna; ARAGAO HOROIWA, Thais; THOMAS, Ranjeny; LANE, Rebecca; ZENG, Biyun

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

☒ Further documents are listed in the continuation of Box C

☒ See patent family annex

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"D" document cited by the applicant in the international application	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 19 February 2025	Date of mailing of the international search report 19 February 2025
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustalia.gov.au	Authorised officer Anthony Lai AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. +61 2 6283 2642

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/AU2024/051401
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KR 20110114952 A (GWANGJU INST SCI & TECH) 20 October 2011 Abstract; Example, Preparation of Anionic Liposomes; Figure 1a-1b	1-32
X	US 20150202285 A1 (The University of Queensland) 23 July 2015 Abstract; pg 25, 30, 33, Table 2B; see pg 51, Example 1, [0171]-[0174]	1-32
A	WO 2011063952 A1 (BRUZZESE, Tiberio) 03 June 2011 Abstract; pg 23, Examples D-E	1-32
A	WO 2022200553 A2 (DANMARKS TENISKE UNIVERSITET; MONTA BIOSCIENCES APS) 29 September 2022 Abstract; pg 88, Example 1	1-32
A	WO 2011098578 A2 (BIONEER A/S) 18 August 2011 Abstract; pg 13, Example 1	1-32

Form PCT/ISA/210 (fifth sheet) (July 2019)

INTERNATIONAL SEARCH REPORT		International application No.	
Information on patent family members		PCT/AU2024/051401	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
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		US 2024197759 A1	20 Jun 2024
WO 2011098578 A2	18 August 2011	WO 2011098578 A2	18 Aug 2011
End of Annex			
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.			